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Pulmon

The Journal of Respiratory Sciences

Editorial

Daily Therapy with Fixed Dose Combinations under RNTCP - Concerns and Apprehensions among Stakeholders in the Background of Global End TB Strategy

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Introduction

Tuberculosis (TB) remains a top infectious killer of men and women and causes persistent human suffering. Globally in 2013, around 9 million people fell ill and 1.5 million died of TB[1]. Our efforts to control TB remain a distant dream because a third of the estimated incident TB goes unnoticed or under diagnosed. Close to half a million MDRTB emerge each year as a result of problems related to diagnosis and treatment. In addition to this, HIV associated TB affects more than one million people every year. We have been able to make considerable success in the diagnosis and treatment of TB in the last two decades, thanks to effort taken by World Health Organization (WHO), World Health Assembly (WHA), International Union Against Tuberculosis and Lung Disease (IUATLD) and various other international and national non-governmental organizations and our Ministry of Health and Family Welfare (MOHFW).

Major advancements include the following:

- Since 1990, the mortality rate of TB has decreased by 40% all over the world
- Between 2000 - 2013, 37 million lives were saved
- Rapid molecular diagnostic tests were developed
- Two new drugs were discovered
- 2% annual reduction in new cases

Now we aim to implement Global End TB strategy[2]. When we implement new policies and programmes there are likely to be many fears and concerns among doctors, health personnel and the public. This is more so when dealing with TB.In this Editorial an attempt is made to highlight the new strategy of daily Fixed Dose Combination (FDC) treatment in TB in the light of Global End TB strategy.

Epidemiology of TB in India

Every year in India around 20, 00,000 people develop TB and more than 3, 00,000 people die of the disease(Table I).Out of the 35385 cases diagnosed as DR-TB in 2013 , only 20753 were put on MDR treatment[2]. TB prevalence per lakh population has reduced from 465 in year 1990 to 211 in 2013. In absolute numbers, prevalence has reduced from 40 lakhs to 26 lakhs. Annual TB incidence per lakh population has reduced from 216 in year 1990 to 171 in 2013[3].

Table I : Burden of TB in India

Burden Indicator	Number in millions (95% CI)	Rate per 100,000 persons
Incidence	2.1 (2.0-2.3)	171 (162-184)
Prevalence	2.6 (1.8-3.7)	211 (143-294)
Mortality	0.24 (0.15-0.35)	19 (12-28)

RNTCP achievements

In our country the Revised National Tuberculosis Control Programme (RNTCP) has met global benchmarks for case detection and treatment outcome. Implementation of universal access to quality TB diagnosis and treatment has helped to engage all care providers. Standards of TB Care in India (STCI) have been laid down to facilitate the above objective. STCI and National Technical Working Group (NTWG) on TB/HIV 2013 has recommended use of daily regimen using FDC of first line drugs[4].

Why does TB need prolonged treatment?

Unlike bacterial infections the treatment of TB is a prolonged one because of the Mycobacterial phenotypes evolved during the process of caseation necrosis. The necrotic, hypoxic microenvironment created during this process facilitates formation of different bacterial population. This is further augmented by tolerance developed for primary drug during the initial phase. Thus the caseating granuloma represents a polymycobacterial infection requiring many months of treatment. The Mycobacterial population in caseating lesions consist of layer after layer of distinct bacterial subpopulations that may also be separated by time and space, each of which may be differentially killed by various antimicrobial drugs dependent on phenotypic drug tolerance or anatomical location. The models developed by Mitchison are often adapted to describe four populations defined by antimicrobial drug efficacy (1) actively growing bacilli mostly killed by INH (2) slow/non-replicating mycobacteria that undergo spurts of metabolism, which are killed by Rifampicin, (3) intracellular bacilli present in the acidic compartments of macrophages or in acidic lung lesions

that are killed by Pyrazinamide, and (4) the dormant mycobcterial persisters found in hypoxic microenvironments with much reduced action of most of the chemotherapeutic agents[5].

Threats faced by RNTCP

People with latent TB infection form a reservoir that sustains the global epidemic.Ageing population with inadequate family and social support is likely to have more chance of active disease. Prevalence of Diabetes Mellitus is on the rise in our community and the chance of break down of old healed lesions is going to be an important health issue in such patients. Consumption of alcohol and alcoholic liver disease are not only issues in disease activation but in treatment-associated hepatitis also. The number of immuno compromised people is on the rise and HIV TB needs special attention. The frequency of tobacco use is high among young individuals and their risk for lung disease including TB is high.

New Global Strategy to End TB

On May 19, 2014, the 67th World Health Assembly adopted WHO's "Global strategy and targets for tuberculosis prevention, care and control after 2015". End TB strategy is marked by well defined milestones and targets. Ending TB epidemic means the following objectives have to be achieved by 2035[6]:

- Fewer than 10 new TB cases occurs per 100,000 population per year
- 90% reduction in TB incidence
- 95% reduction in TB deaths

Why daily treatment?

India bears one-third of Global TB burden and we were using intermittent regimen under DOTS so far. But recent studies have shown that relapse rates are much lower (5%) for daily regimen when compared with the intermittent regimen (10%)[7]. Moreover, In India, the prevalence of INH resistance is very high (40%), Hence the relapse rate can be as high as 20%[7]. To ensure proper treatment, WHO and IUATLD decided to introduce 4 and 3 - drug FDCs for simplifying the drug prescription. It may also make management of drug supply more efficient. An additional advantage is that it may limit the risk of drug-resistant tuberculosis arising as a result of inappropriate drug selection and monotherapy[8]. Thus it can provide a simple approach to delivering the correct number of drugs at the correct dosage. Complete treatment is delivered by altering the number of tablets according to the patient's body weight[8].

Table II : RNTCP Daily Treatment Regimen

Type of TB Case	Treatment Regimen	
	Intensive Phase	Continuation Phase
New	2HRZE (56 doses = 8 X 7 days/ week or 28 X 2)	4HRE (112 doses = 16 weeks X 7days/week or 28 X 4)
Previously treated	2HRZES + 1HRZE (84 doses = 12X7 or 28X3)	5HRE (140 doses = 20 weeks X 7days/week or 28X5)

TB cases under RNTCP is now classified under two categories: new cases (defined as those that have not received ATT in the past) and previously treated cases (Table II).

Table III : Recommended FDC pills for daily use in India (Drugs and their Strength in mg)

4-FDCs for Intensive Phase (IP)	
HRZE	H (75) + R (150) + Z (400) + E (275)
3-FDCs for Continuation Phase (CP)	
HRE	H (75) + R (150) +E (275)

4 drug FDC, each with 75 mg INH,150 mg Rifampicin, 400 mg Pyrazinamide and 275 mg Ethambutol is recommended for Intensive phase. For continuation phase, 3 drug FDC each with INH 75, Rifampicin 150 and Ethambutol 275 is recommended. As per weight band, the number of tablets vary. Paediatric patients may continue current therapy only (Table III).

Table IV : Daily dosage schedule for Adults

Weight category	Number of tab in IP	Number of tab in CP	Inj. SM
25-39 kg	2	2	0.5 gm
40-54 kg	3	3	0.75
55-69 kg	4	4	1 gm
>70	5	5	1 gm

Since majority of our patients belong to the weight band 40 to 54kg category, most cases can be treated with 3 tabs daily (Table IV).

Implementation of therapy

For daily therapy RNTCP has retained the previous concept of 'One Patient One Box'. Patient wise box consist of blister packs of

Schedule drug 9 for IP and Schedule 10 for CP packed in separate laminated pouches. The number of blister packs in each pouch will be as per the weight band. Drugs are packed to cover 4 weeks of treatment, i.e 28 days, dispensed on a daily basis. The effective number of doses the patient would be receiving in a month would be 28. MOHFW is likely to purchase and distribute the drugs[9]. There is also provision for loose drugs for weight bands outside the given range.

Direct Supervision and Cost-effectiveness

We have been using Fixed Dose Combination of INH and RMP along with individual formulations of ETB and PZA in the past. Our experience shows that with the available combinations we were able to treat most of the fresh patients as the maximum pill burden was only 4 for most of the TB cases. There was no confusion regarding the drug types. We also believe the bioavailability of drugs used by us previously were of standard formulations. The critical issue was direct supervision.

We should think twice before accepting the new strategy that can involve huge investment on the part of the society when we have alternatives of equal efficiency. Our experience showed that the critical problem with National Tuberculosis Programme (NTP) was poor adherence and monotherpy. The solution suggested in RNTCP was direct supervision and Fixed Dose combination of INH and RMP. But the management was complicated because of the various categories and related prescriptions. Now the categories have been simplified but implementation has been made complex by adding more drugs in the formulations and diluting the vital component of direct supervision. All of us agree that adding more drugs to FDC is more expensive and should provide more benefit to justify the investment. Only selected pharmaceuticals have the capability of producing FDC of 3 or 4 primary anti TB drug of acceptable bioavailability. If proper direct supervision is available then the available drug combinations can also produce the same outcome with less additional cost.

Clinical and Laboratory Follow up of patients

Patients are followed up clinically as well as by laboratory tests.The follow up will be on a monthly basis at the nearest health facility and would review improvement in symptoms,weight gain, signs of adverse reactions to drugs and appropriate management of co-morbid conditions.

Laboratory investigations : Necessary laboratory investigations need to be done to assess progress and prognosis of the disease and to manage co-morbidities or adverse reactions. Deterioration at the end of IP needs detailed evaluation for non response and appropriate action.

Prevention and management of adverse reactions

Most first line drugs are well tolerated. But a few who develop adverse drug reactions(ADR) need to be identified early and managed. A serious event or reaction is any untoward event at any time during treatment which resulted in Death, Drug induced hepatitis; required inpatient admission and treatment resulted in termination of drugs due to ADR.

Critical activities required for maintaining health

Considering the increasing prevalence of risk factors for triggering infection to disease and treatment completed cases to relapse, prompt evaluation and appropriation is required for those with risk factors.A delayed diagnosis of Pulmonary TB may result in structural damage of the lung leading to cavity, fibrosis and bronchiectasis. Most patients with such structural damage and persistent smoking habit are at risk of developing recurrence of symptoms due to bronchitis, lung cancer and relapse with drug resistance.

Understand the strengths and weaknesses of current therpy

We should accept the fact that we have not been able to eradicate the Tubercle bacilli from our body with the current therapeutic agents. Among the currently used first line drugs INH and RMP acts on most of the bacterial populations. Extracellular rapidly multiplying organism in the caseous material and cavitary wall form the major bacterial phenotypes. Intracellular organism though smaller in number is acted upon by Pyrazinamide . Completely dormant organism is acted upon by none of the currently used drugs. They are taken care of by the individual's defence mechanism. As evidenced in TB patients that relapse during treatment of drug-sensitive mycobacteria, the host immune system cannot effectively eliminate these residual dormant bacilli that are not killed by chemotherapy. Therefore, although achieving a clinical cure, the current anti-TB standard regimen does not necessarily achieve a bacteriological cure. In other words, current therapy does not completely eradicate all bacilli from the body, but allows the infection to be contained effectively for long periods of time.

Move forward cautiously till we get an ideal solution that is not too far

Considering the vast interests and investments from the international and national agencies, it is not too far to achieve our targets. But management of TB is not simple. RNTCP guidelines need to be strictly implemented. Current diagnostic algorithm involves combination of both sensitive and specific tests: sputum examination and radiology evaluation. This is important and relevant for smear posi-

tive and smear negative cases. Structural damage and its extent at the time of diagnosis and extent of healing at the time of completion of chemotherapy has a bearing on subsequent lung health. Smear positive fresh case of PTB can be treated in any facility as per guidelines. Extra pulmonary TB needs regular follow up evaluation. Smear negative cases, PTB with complications, drug intolerance, relapse, retreatment and resistant cases need specialist attention.

Hence playing with therapeutic agents need extreme caution. RNTCP with DOTS has evolved from lessons learned from the NTP. Later we formulated the standards of TB care for India. Now we are in the process of simplifying TB care by introducing daily treatment as FDC. If we have to achieve the mile stones and targets set for END TB strategy the answer is not FDC. We need point of care diagnostic tests and very effective and safe new sterilising drug combinations that may eradicate the microbe in a few weeks time and prevent drug resistance. We need to move very cautiously as well as very fast. The scarce resources available for achieving the above targets need to be conserved. Otherwise mycobacteria will find new ways to maintain its habitat in our body which it occupied from the beginning of human civilization.

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Review Article

Molecular Biology of Lung Carcinoma

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Lung cancer is one of the commonest cancers and cause of cancer related deaths all over the world. It accounts for 13 per cent of all new cancer cases and 19 per cent of cancer related deaths worldwide. There were 1.8 million new lung cancer cases estimated to occur in 2012. In India, lung cancer constitutes 6.9 per cent of all new cancer cases and 9.3 per cent of all cancer related deaths in both sexes; also it is the commonest cancer and cause of cancer related mortality in men. Despite tremendous progress in therapeutic modalities, lung carcinoma still represents a malignancy with poor prognosis when detected at advanced clinical stage.[1-3]

Approximately 85–90% of all cases of lung cancer are carcinomas of non-small cell type. These tumors can be further classified into several major histological subtypes, including adenocarcinoma, squamous cell carcinoma, large cell carcinoma, adenosquamous cell carcinoma, and sarcomatoid carcinoma.[4]

Table 1 : IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens

Preinvasive lesions
Atypical adenomatous hyperplasia
Adenocarcinoma in situ (≤3 cm formerly BAC)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Minimally invasive adenocarcinoma (≤3 cm lepidic predominant tumor with ≤5 mm invasion)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Invasive adenocarcinoma
Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
Acinar predominant
Papillary predominant
Micropapillary predominant
Solid predominant with mucin production
Variants of invasive adenocarcinoma
Invasive mucinous adenocarcinoma (formerly mucinous BAC)
Colloid
Fetal (low and high grade)
Enteric

BAC, bronchioloalveolar carcinoma; IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society.

All cancers arise as a result of changes that have occurred in the DNA sequence of the genomes of cancer cells- Mutations.

'Driver' mutation's confer growth advantage on the cells carrying them and have been positively selected during the evolution of the cancer. They reside, by definition, in the subset of genes known as 'cancer genes'.

'Passenger mutations' do not confer growth advantage, but happened to be present in an ancestor of the cancer cell when it acquired one of its drivers.

'Signal Transduction' The intracellular transfer of information (biological activation/inhibition) through a signal pathway. In each signal transduction system, an activation/inhibition signal from a biologically active molecule (hormone, neurotransmitter) is mediated via the coupling of a receptor/enzyme to a second messenger system or to an ion channel. Signal transduction plays an important role in cell functions like cell proliferation.

In the era of molecular and personalized therapeutics, the ability to detect multiple driver mutations in lung adenocarcinoma has revolutionized the medical management of this disease and multiplexed testing for all common driver mutations will provide physicians with a more precise guide for therapy. The common mutations detected in many reviews include mutations involving KRAS, EGFR, ERBB2 (HER2), BRAF, PIK3CA, AKT1, MAP2K1, NRAS and EML4–ALK rearrangement. These can be screened using standard multiplexed assays and FISH. Driver mutations were detected in 60% of tumors. The incidences of mutations were as follows: KRAS- 25%, EGFR - 23%, ALK rearrangements- 6%, BRAF-

3%, PIK3CA - 3%, MET amplifications - 2%, ERBB2- 1%, MAP2K1 - 0.4%, NRAS 0.2%.Approximately 36.4% of lung adenocarcinomas do not harbor currently detectable mutations. It is noteworthy that 95% of molecular lesions were mutually exclusive. Table 2 [5, 6] EGFR may also serve as a prognostic factor, in addition to its role as a predictive factor, as patients-bearing EGFR mutations have shown favorable clinical outcomes even with conventional chemotherapy.[7]

EGFR genotype was more useful than clinical characteristics for selection of appropriate patients for consideration of first-line therapy with an EGFR TKI. EGFR mutations are generally associated with sensitivity to TKI therapy. Both retrospective and prospective studies have demonstrated that lung adenocarcinoma patients carrying such an EGFR mutation and who were treated with TKIs had significantly higher response rates and longer progression-free survival than patients without an EGFR mutation.[6]

EGFR mutations in lung cancer

EGFR- (EGFR; ErbB-1; HER1)

EGFR is located at chromosome 7 p11.2, spans about 200 kb, and contains 28 exons. EGFR gene encodes a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation.

Tyrosine kinases are enzymes that catalyze the transfer of the γ phosphate group from adenosine triphosphate to target proteins. They play an important role in diverse normal cellular regulatory processes. Tyrosine kinases can be classified as receptor protein kinases and nonreceptor protein kinases. The receptor tyrosine kinases are membrane-spanning cell surface proteins that play critical roles in the transduction of extracellular signals to the cytoplasm. There are approximately 60 receptor tyrosine kinases that have been identified, and they are divided into some 20 subfamilies as defined by receptor and/or ligand. They are characterized by immunoglobulin-like sequences in their amino-terminal extracellular domains, a lipophilic transmembrane segment, and an intracellular carboxyl-terminal domain that includes the tyrosine kinase catalytic site. Nonreceptor tyrosine kinases, on the other hand, relay intracellular signals.[9]

Table 2 : Driver mutations in NSCLC

Unknown	36.4%
KRAS	25%
EGFR	23%
EML4ALK	6%
BRAF	3%
PIK3CA	3%
MET	2%
ERBB2	1%
MAP2K1	0.4%
N RAS	0.2%

Ligand binding induces dimerization of these receptor tyrosine kinases, resulting in autophosphorylation of their cytoplasmic domains and activation of tyrosine kinase activity. Multiple cytoplasmic signaling pathways, including the Ras/Raf mitogen-activated protein kinase pathway, the phosphoinositol 3'-kinase/Akt pathway, the signal transducer and activator of transcription 3 pathway, the protein kinase C pathway, and scaffolding proteins may then be activated. Intracellular mediators in these pathways transduce signals from membrane receptors through the cytosol and into the nucleus, culminating in altered DNA synthesis and cell division as well as effects on a variety of biological processes, including cell growth, migration, differentiation, and death.[9]

Mutations in EGFR gene are associated with uncontrolled cell proliferation. Epidermal growth factor receptor (EGFR)-TK is a transmembrane receptor TK that is overexpressed or aberrantly activated in the most common solid tumors, including non-small cell lung cancer and cancers of the breast, prostate, and colon. Uncontrolled activation of the EGFR-TK enzyme results in autophosphorylation, which drives signal transduction pathways leading to tumor growth and malignant progression.

The incidence of EGFR mutations in unselected tumors with non-small cell histology ranges from 10 to 50%, also 90% incidences seen in lung adenocarcinomas, are preferentially observed in a specific subset of patients: females of East Asian ethnicity who have never smoked and who have adenocarcinoma with lepidic growth pattern (formerly classified as bronchioloalveolar carcinoma).

Table 3 : Diagnostic modalities to identify mutations in lung cancer

Selected therapeutically relevant genomic alternation in NSCLC	Sanger sequencing	Immuno histo chemistry	Fluorescence In Situ Hybridisation	Multiplex Hotspot Mutation testing	Multiplex Sizing Assays	Next-Generation sequencing
Rearrangements ALK ROS1 RET NTRK		for ALK and ROS1, FISH confirmation required	+ +			+
Point Mutations EGFR KRAS ERBB2(HER2) MAP2K1(MEK) BRAF PIK3CA AKT	+	+ (EGFR L858R)		+		+
Insertion or Deletions EGFR ERBB2(HER2)	+	+ (EGFR Exon 19 deletion)			+	+
Amplification MET Loss PTEN		+ (MET amplification requires FISH confirmation)	+			+
Non-Recurrent Genomic Alternation Involving the above genes and other potentially relevant oncogenes and tumour						+

In adenocarcinomas, the majority of mutations have been identified in exons 18–21 of the EGFR gene. These mutations can be roughly classified into three major categories: in-frame deletions in exon 19, insertion mutations in exon 20, and missense mutations in exons 18–21. The most frequent mutations were located at exon 19 and exon 21. There are over 20 variant types of exon 19 deletions, with the most common including delE746-A750, delL747- T751insS, and delL747-P753insS. L858R, in exon 21, is the second most frequent mutation.

The *exon 20 insertions* frequently associated with EGFR-TKI non-responsiveness, The most important mutation in exon 20 is T790M, which is associated with a small fraction of adenocarcinomas with primary resistance to EGFR TKI and over one-half of the patients with acquired resistance to EGFR TKI.

EGFRvIII Mutation

EGFR variant III (EGFRvIII), a mutation resulting from an in-frame deletion of exons 2–7 of the coding sequence , has been associatedwith a subset of squamous cell lung cancers.EGFRvIII hasbeen identified in an array of human solid tumors, including glioblastoma, breast cancer, ovarian cancer,prostate cancer, and lung caner. Although EGFRvIII fails to bind EGF, its intracellular tyrosine kinase is constitutively activated, allowing the receptor to undergo tyrosine autophosphorylation. EGFRvIII-bearing squamous cell carcinomas were reportedly insensitive EGFRTKI Inhibitors

A comprehensive literature review by Yamamoto et al indicated that 569 mutations were found in 2880 lung cancer patients (20%). The distribution of EGFR mutations was as follows: 48% in exon 19, 43% in exon 21, 4% in exon 20, and 3% in exon 18. EGFR mutations, except EGFRvIII, are rarely found in squamous cell and large cell carcinomas, thus EGFR TKI therapy may not be a relevant therapy for patients with those tumors. In a large series of lung carcinomas investigated for the presence of EGFR mutations in exons 18, 19, and 21, no EGFR mutations were found in the 454 squamous carcinomas and 31 large cell carcinomas investigated. In contrast, EGFR mutations were found in 10% of 375 adenocarcinomas and in 26% of the 86 cases designated as bronchioloalveolar carcinomas.

The most commonly used method to detect EGFR mutations is direct sequencing. Formalin-fixed and paraffin-embedded tissue is perfectly suitable for fluorescence in situ hybridization (FISH) and DNA-based tests, but tissue preservation is critical for a successful test. Decalcified and ethanol-fixed tissue, as well as tissues containing abundant necrosis, should be avoided.

EGFR targeted therapy approaches

Activating mutations in oncogenes have been described as an 'Achilles heel' in cancer, coining a term of 'oncogene addiction'. Dependency of a tumor on a specific oncogene renders these malignancies potentially sensitive to inhibitors that preferentially target the altered oncogene and thereby abrogates the tumor-promoting properties of it. In fact, sudden cessation of signaling can induce cell death.

To target EGFR the two major approaches are: (1) prevent ligand binding to the extracellular domain with a monoclonal antibody and (2) inhibit the intracellular tyrosine kinase activity with a small molecule TKI.

Monoclonal Antibodies

Monoclonal antibodies, such as cetuximab and panitumumab, are either chimeric mouse-human or fully humanized antibodies targeting the EGFR extracellular domain, leading to blockade of ligand activated signal transduction and receptor dimerization. The binding of the antibody initiates EGFR internalization and degradation, which leads to signal termination. However, this class of treatment only inhibits ligand-dependent activation of EGFR and not autophosphorylation of the tyrosine kinase domain via constitutive activation. These mutations may still activate the downstream pathways, and upregulate cell cycle progression, cell growth, and angiogenesis.

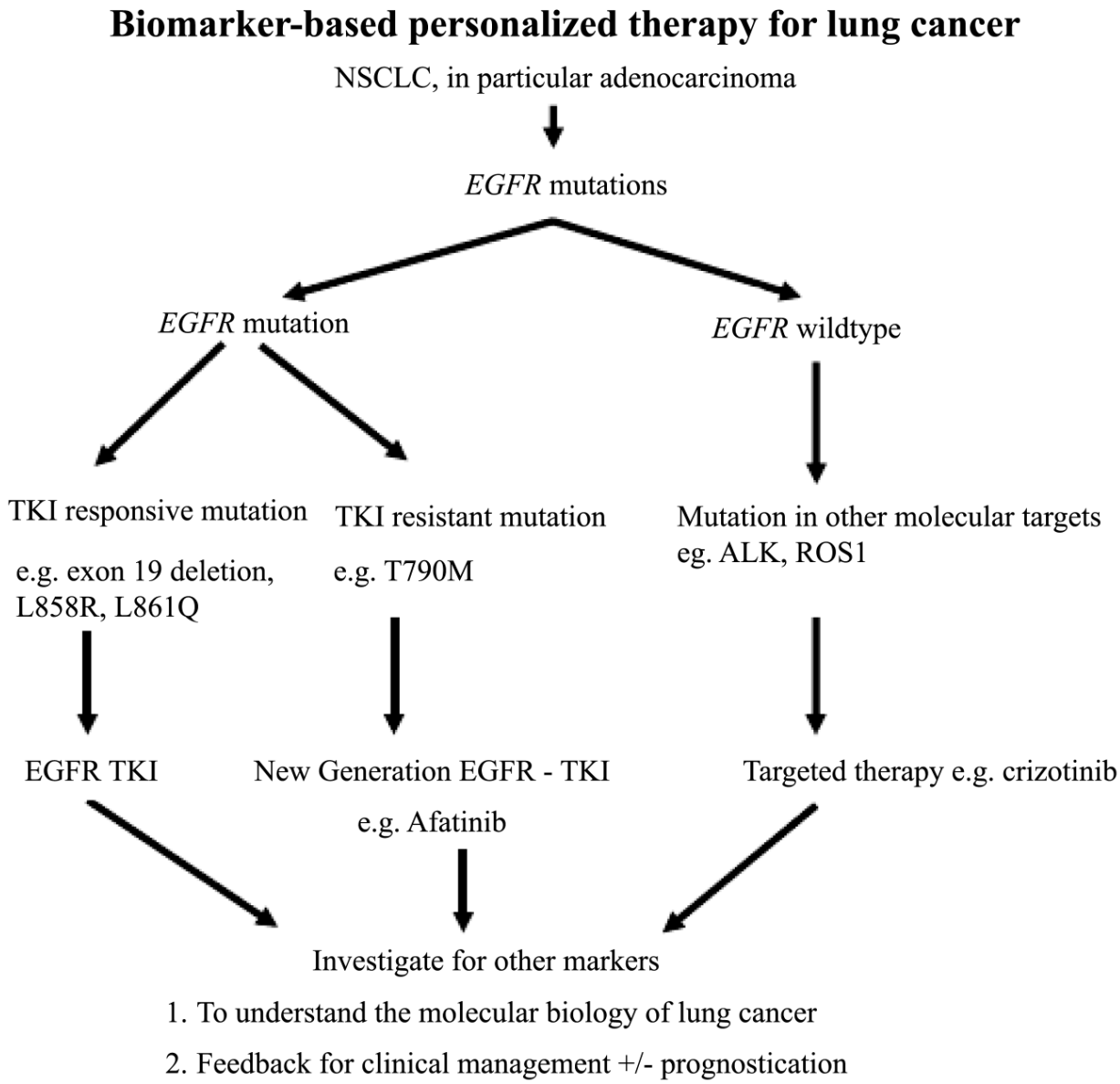
Tyrosine Kinase Inhibitors

TKIs are synthetic small molecules that block the magnesium-ATP-binding pocket of the intracellular tyrosine kinase domain. TKIs prevent the intracellular tyrosine kinase domain of the EGFR from autophosphorylation through binding to its ATPbinding site. Several TKIs, such as gefitinib and erlotinib, are specific for EGFR, whereas others inhibit other receptors in addition to EGFR, such as ERBB2 and VEGFR2. TKIs block ligand-induced receptor autophosphorylation by binding to the tyrosine kinase domain and disrupting tyrosine kinase activity, thereby abrogating intracellular downstream signaling.

Resistance Pathways

De Novo / primary Resistance to EGFR TKIs

De novo resistance to EGFR TKI treatment in EGFR mutant NSCLC patients have been detailed as, due to (1) secondary alterations in EGFR that prevent inhibition of EGFR by an EGFR TKI (drug resistant EGFR mutation), and (2) additional genetic alternations other than EGFR mutation that can co-occur with an EGFR activating mutation in EGFR mutant NSCLC cells.



Majority of *mutations in exon 20* are resistant to EGFR TKI treatment, an insertion or duplication in exon 20 or an EGFR T790M mutation. Insertion mutation in exon 20 renders the EGFR 100-fold less sensitive to TKIs when compared with the sensitizing mutations. The most commonly found mutations associated with TKI drug sensitivity include exon 19 deletions downstream of the lysine residue at position 745 (DE746-A750), point mutations in exon 21 (L858R and L861Q and L861R), in exon 18 (G719A/C/S), and in exon 20 (V765A, T783A, and S768I).

Primary TKI resistance may also be mediated by the presence of *other genetic alterations* that affect signaling downstream from EGFR, such as mutation of KRAS, PIK3CA, and loss of PTEN expression.

The wild-type EGFR appears to be a significant marker for the primary EGFR TKI resistance. Tumors with wild-type EGFR often harbor somatic mutations in other genes that affect the key pathways in lung adenocarcinoma. Thus, primary drug insensitivity is likely linked to the absence of drug-sensitizing mutations in EGFR and is more likely to be a result of mutations in other genes.

KRAS Mutations : KRAS has a key role in the EGFR signaling network. It has an important role in EGFR downstream signaling. Some tumors harbor somatic mutations in exon 2 of KRAS that lead to constitutive activation of the RAS pathway. This pathway is identical to the EGFR pathway targeted by TKIs in adenocarcinomas. An activating mutation of KRAS is present in 25–

35% of TKI non-responsive cases, since in these cases inhibiting the TK will not downregulate the consecutively activated KRAS in the downstream pathway. . EGFR and KRAS mutations are rarely detected in the same tumor, suggesting that they may perform functionally equivalent roles in lung tumorigenesis. KRAS mutation is a negative predictor of response to anti-EGFR monoclonal antibodies and is also an important mechanism of resistance to TKIs in lung adenocarcinoma. A meta-analysis by Linardou et al provided empirical evidence that somatic mutations of the KRAS oncogene are highly specific negative predictors of response to single-agent EGFR TKIs in advanced lung cancers, mostly adenocarcinomas.

BRAF Mutations : are found in 1–3% of lung cancers, most of which are adenocarcinomas. Both KRAS and BRAF genes are part of the signaling cascade for the EGFR family proteins. The BRAF protein is a serine/threonine protein kinase that is activated by KRAS in a GTP-dependent manner.

MET Amplification MET also contributes to primary and acquired resistance to EGFR TKIs. MET is located on chromosome 7q21, which encodes the tyrosine kinase, hepatocyte growth factor receptor. MET amplification occurs in both squamous cell carcinoma and adenocarcinoma.

Acquired / Secondary EGFR Mutations in Lung Adenocarcinomas after EGFR Receptor Inhibitor Treatment

Despite the remarkable response of EGFR mutant lung adenocarcinomas to TKIs, many of these tumors recur and eventually develop secondary evolved resistance. These resistant tumors have commonly acquired TKI treatment related EGFR mutations.

One of these mutations is, as previously mentioned , located in exon 20 of the EGFR gene and replaces methionine for a threonine (T790M), resulting in increased ATP affinity. .

Another well-defined separate mechanism of acquired resistance to EGFR TKIs is the amplification of MET, the gene encoding a different membrane-bound RTK. MET amplification occurs regardless of the T790M status, and its amplification in cells originally dependent upon mutant EGFR illustrates a phenomenon that can be described as "kinase switch," in which surviving EGFR mutation–positive oncogenic cells exposed to prolonged action of EGFR kinase inhibition develop resistance by becoming dependent on another kinase, such as MET. Analysis of tumor samples and follow-up studies from multiple independent patients with EGFR mutation–positive NSCLC suggests that the prevalence of MET amplification may be closer to 10%.

Other rarer forms of acquired resistance that have been described but not completely understood include activating PIK3CA mutation, transformations to SCLC, activation of insulin-like growth factor receptor pathway, and epithelial-mesenchymal transition (EMT).The exact frequencies of these mechanisms have not been completely established.

EML4–ALK rearrangement:

The ALK gene encodes a receptor tyrosine kinase found in a number of fusion proteins consisting of EML4–ALK fusion formed as the result of a small inversion within the short arm of chromosome 2 that joins intron 13 of ECHINODERM MICROTUBULE ASSOCIATED PROTEIN-LIKE 4 (EML4) to intron 19 of ALK [inv(2)(p21;p23)], generating an oncogenic fusion encoding a constitutively activated protein tyrosine kinase. A subset of lung adenocarcinoma cases harbor within the genome this transforming fusion gene, EML4–ALK.

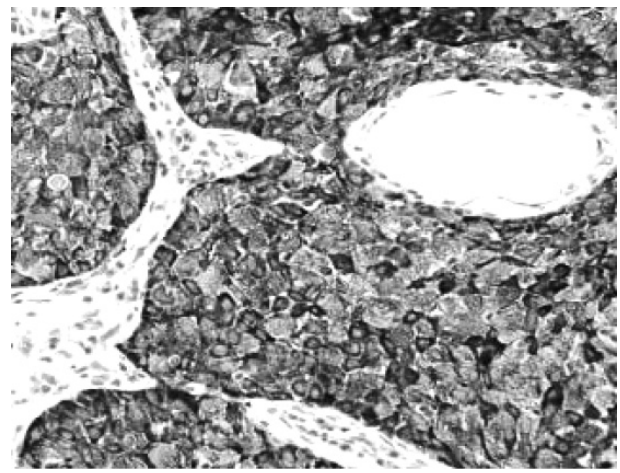


Figure 1 : IHC for ALK

The EML4–ALK fusion is a rare abnormality detected in 3–13% of patients with adenocarcinomas. The most common fusion results from the joining of exons 1–13 of EML4 to exons 20–29 of ALK. At least seven EML4–ALK variants (V1–V7) have been identified in lung adenocarcinomas. All seven variants are formed through the fusion of the intracellular tyrosine

kinase domain of ALK with a variably truncated EML4 gene. Activated ALK is involved in the inhibition of apoptosis and the promotion of cellular proliferation through activation of downstream PIK3CA/AKT1- and MAPK1-signaling pathways.Fusion of the EML4–ALK gene and its associated EML4–ALK product may further lead to constitutive activation of the RAS/RAF1/MAP2K1/MAPK1 pathway. The key downstream effectors on the ALK pathway include the RAS-activated protein, extracellular signal regulated kinase (MAPK1), phosphoinositide 3-kinase (PIK3CA), and STAT3 signaling pathways.RAS/ MAP2K1/MAPK1 pathways are critical for cell proliferation, whereas the PIK3CA/AKT1 and STAT3 pathways are important for cell survival.

It has been reported that although ALK-fusion positive lung cancers are resistant to the EGFR TKIs, gefitinib, and erlotinib, they are sensitive to small molecule TKIs against ALK. ALK TKIs (ALK TKI), including crizotinib, are effective treatments for patients with ALK-fusion cancers.

However, despite these remarkable initial clinical responses, these cancers eventually developed resistance to crizotinib, usually within 1 year, thereby limiting the potential clinical benefit of this drug. Katayama et al found that cells resistant to intermediate doses of crizotinib develop either amplification of the EML4–ALK gene or a gatekeeper mutation, L1196M, within the kinase domain.

However, unlike EGFR mutation, ALK rearrangement was not a favorable prognostic factor. Dual-color–split-apart FISH is the recommended method for the EML4–ALK test.

Practical considerations for molecular testing in lung carcinomas

The most common method in practice for identification of EGFR mutation is PCR based techniques , we can specifically look for the commonest mutation in exon 19 & exon 20.multiplex PCR is another method where we can look for multiple mutations commonly in Exon 19, 20 & 21.To look for amplifications like MET amplification FISH is the preferred method . Pan-EGFR immunohistochemistry is not recommended for detection of mutations. Antibodies to specific common mutations are preferred over Pan EGFR.

Conclusions

In summary, EGFR and ALK TKI therapy has provided a novel treatment modality for patients diagnosed with adenocarcinoma lung .But, primary and acquired resistance to targeted therapy influence the final outcome of treatment . Identification of the specific molecular alterations along with a growing understanding of the mechanisms of pharmacotherapy and the evolution of molecular resistance, lung cancer treatments will be specifically tailored for the individual patient based on the presence or absence of critical molecular alterations.

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Original Article

DECAF Score as an Outcome Prediction Tool in COPD Exacerbation

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Abstract

Background & Objective : Exacerbations greatly influence the clinical course in COPD. A simple tool - DECAF Score (Dyspnoea, Eosinopenia, Consolidation, Acidemia, atrial Fibrillation) - has been introduced recently for predicting the hospital mortality in exacerbations of COPD. This study is conducted to find the usefulness of the DECAF score in our population.

Materials & Methods: A prospective cohort study was conducted in patients with COPD exacerbation in Institute of Chest Diseases, Govt. Medical College, Kozhikode. The DECAF score was calculated for each patient, and were categorized in to different risk groups. Patients were followed up in the hospital. Statistical analysis was done to find out any correlation between DECAF score and the clinical outcome.

Results : A total of 118 patients were included in the study. Based on the respective DECAF score, 50.84% of the patients were grouped in low risk category, while 22.03% and 27.11% of patients were included in intermediate and high risk categories respectively. The mean number of days of hospital stay of the study population was 8.34 days. The mean score of those patients who got discharged in less than 10 days (n- 84) was 0.96 and who stayed more than 10 days (n-34) had a mean score of 3.09. 18 patients were admitted in ICU, of which 88.9% of patients (n-16) belonged to high risk group. 15 patients who required assisted ventilation had a mean score of 4.13. 92.3% of all the deaths occurred in high risk group. All the observations were found to be statistically significant ($p < 0.05$).

Conclusion : DECAF score can be used to predict the outcome of COPD exacerbation. It can be used in the initial triage of patients and thus helps the physician to decide the site and level of care needed in AECOPD.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) continues to be an important cause of morbidity, mortality, and health-care costs worldwide. Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are periods of acute worsening which greatly affect the health status of patients with an increase in hospital admission and mortality. The greater the severity of COPD, the greater the likeliness of hospital admission and also of death. A simple tool -DECAF Score- has been introduced recently for predicting the hospital mortality in exacerbations of COPD[1]. This scoring system uses indices that are routinely available at

the time of hospital admission. This simple scoring system may help clinician to triage the patients, categorize them into different risk strata and plan different management levels for optimum care. But validation of the DECAF score in our population is required.

Materials and Methods

This Prospective Cohort Study was conducted in the Institute of Chest Diseases, Govt. Medical College, Kozhikode, from February 2013 to January 2014. Consecutive patients admitted with Exacerbation of COPD (in whom persistent airflow obstruction, post bronchodilator FEV1/FVC < 0.70, was confirmed when clinically stable) were enrolled in the study. Patients with primary reason for admission other than exacerbation of COPD, co-morbidities which may significantly affect outcome, like recent Myocardial infarction, were excluded. Following a detailed medical history and physical examination, routine investigations like Complete haemogram including Absolute Eosinophil Count (AEC), PA Chest Radiograph, ABG and ECG were performed. Based on these data, the DECAF score (Table 1) was calculated as follows :

Table 1 : Calculation of DECAF score:

Variable	Score
Dyspnea limiting the patient to home (MRCD 5)	
Independent in bathing and/or dressing (5a)	1
Requires assistance with bathing AND dressing (5b)	2
Eosinopenia (= 0.05 X 10^9/L)	1
Consolidation (on chest x-ray)	1
Acidemia (pH <7.35)	1
Atrial Fibrillation	1

Dyspnea was assessed by asking the patient to rate the level of breathlessness on a good day within the preceding 3 months, not at the time of admission and the level of dyspnea was scored (Table 2) according to the extended Medical Research Council Dyspnea (eMRCD) scale.

Table 2 : Dyspnea scale

Limitation due to Breathlessness	eMRCD Scale
Breathless only with strenuous exercise	1
Breathless when hurrying on the level or walking up a slight hill	2
Walks slower than peers, or stops when walking on the flat at own pace	3
Stops after walking 100m, or for a few minutes, on the level	4
Too breathless to leave the house-	5
& independent in washing and / or dressing	5a
& dependent in washing and dressing	5b

The patients were categorized in to different risk groups based on the total score as shown in Table 3.

Table 3 : DECAF Risk categories

Category	DECAF Score
Low	0 - 1
Intermediate	2
High	≥ 3

All the patients were managed as per standard treatment guidelines for COPD exacerbation. Patients were carefully followed up in the hospital. Patient's clinical course in terms of total number of days of hospital stay, need for assisted ventilation, ICU admission and final outcome were noted. The findings were tested against the DECAF score to find out any correlation, using statistical software SPSS ver.16.

Observations

A total of 118 patients were included in the study, of which 111 were males and 7 females. Mean age of the study population was 63.32 years, majority (63.56%) were aged > 60 years, 33.9% were aged between 41 to 60 years and 2.54% aged ≤ 40years. 82 patients had significant smoking score of 20 pack years. 5 were non smokers.

DECAF Score

38 patients had a baseline dyspnea of 5b. Of the total 118 admissions, 55 patients (46.6%) had type 2 respiratory failure with respiratory acidosis. Eosinopenia was noted in 20 (16.9%) patients. 15 (12.7%) patents had evidence of consolidation in chest radiograph. There were 9 cases with AF. Majority of patients (33.1%) had score 0 at admission. The highest score observed was 5, in two of the patients.

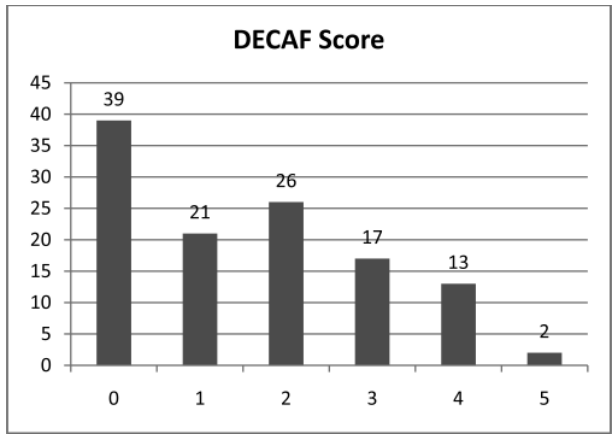


Table 4 : DECAF risk categorization

Category	Number of Patients
Low	60
Intermediate	26
High	32

50.84% of the patients were in low risk group, while the intermediate and high risk categories included 22.03% and 27.11% of patients respectively.18 patients (15.3%) required ICU admission, of which 15 patients required assisted ventilation, either non invasive or invasive. The mean hospital stay was 8.34 days. The total mortality was 13 (11%) and the rest 105 patients were discharged after stabilization of respiratory status. Statistical analysis was done using Pearson chi-square test and T test and p value of less than 0.05 was taken as significant.

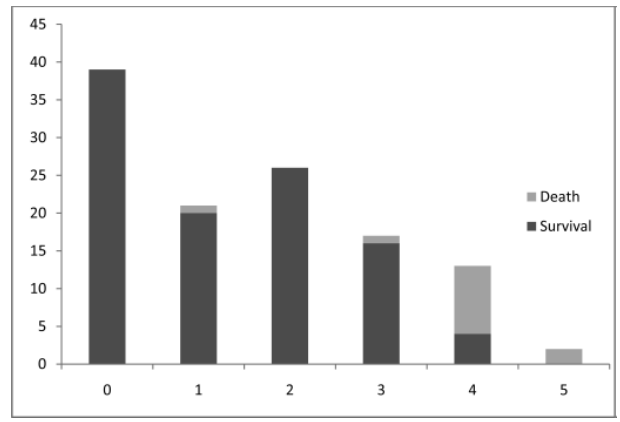
The mean DECAF score of patients who got discharged within 10 days (n-84) was 0.96 and who stayed more than 10 days (n-34) had a mean score of 3.09. There was statistically significant ($p<0.05$) increase in number of days of hospitalization in each of the risk categories.

ICU Admission & Assisted Ventilation

Table 4 : ICU & Assisted Ventilation

	ICU Admission		Assisted Ventilation	
	Yes	No	Yes	No
Low	0	60	0	60
Intermediate	2	24	0	26
High	16	16	15	17

Out of 118 patients, 18 were admitted in ICU, had a mean DECAF score of 3.83, of which 16 patients were belonged to high risk group, 2 patients from intermediate and none from low risk group. None of the patients with low or intermediate risk, required assisted ventilation. All of the 15 patients who required assisted ventilation had a mean score of 4.13, while the mean scores of patients who did not require ICU care or assisted ventilation, were 1.17 and 1.20 respectively. All these observations had a p value of < 0.05.



Mortality trend of DECAF score

It is evident from the above illustrations that, as the DECAF score increases the mortality also increases. The mean score of patients, who got discharged (n-105) after stabilization, was 1.30 and that of patients who died due to exacerbation (n-13) was 3.85. 92.3% of all the death occurred in high risk group, where as only one death has occurred in low risk group. All the observations are found to be statistically significant ($p<0.05$).

Discussion

Exacerbations are a significant component of the clinical course in COPD. Furthermore, as COPD progresses, exacerbations become more frequent. Exacerbations significantly increase the cost for the treatment of COPD. Clinical studies have reported a high mortality rate in patients admitted to the hospital with an acute exacerbation of COPD[2-6]. Several studies have identified the risk factors associated with increased mortality.

The GOLD and ATS recommend that a patient's perception of dyspnea be included in any new staging system for COPD[7,8]. 84.6% of patients who died in our study had a basal dyspnoea that limited them physically to get confined to their home and to get assistance in their daily routine.

Acidosis is associated with increased mortality and higher need for intubation[9,10]. Cardiac arrhythmia is an important cardiovascular comorbity of COPD, which can increase the inpatient costs and mortality during exacerbation[11]. Two major hypotheses for arrhythmogenesis in COPD have been proposed. The electropathy due to hypoxaemia, hypercapnia and acid–base disturbances are the three main, COPD-related, arrhythmogenic triggers. Second is Autonomic neuropathy[12]. Atrial fibrillation (AF) and COPD frequently coexist and complicate treatment of both conditions. Changes in blood gases, abnormalities in pulmonary functions, and hemodynamic changes resulting from pulmonary hypertension can lead to the development of AF. AF has been proven to be one of the main cardiovascular risk factors for in hospital mortality in AECOPD[13]. The incidence of AF in our study is 7.62%.

Acute infection can cause eosinopenia[14] through several mechanisms, such as peripheral sequestration of eosinophils in inflammatory sites, suppression of the egress of mature eosinophils from the bone marrow, suppression of eosinophil production[15], and adrenal glucocorticoids and epinephrine mediated mechanisms in acute stress. It has been shown that low eosinophil cell count at admission and during the first 7 days as a prognosis marker of mortality in medical ICU[16]. The most acceptable cutoff for the eosinophil count in adults as a predictor of mortality is 40-50 cells/ μL[16-18].

Steer J et al[13] has come up with a new scoring system which incorporates all the above mentioned parameters of increased mortality and morbidity in AECOPD, which is evaluated in our study as an outcome predictive tool and is found to be correlating well with the clinical course of the patients in hospital. The DECAF score is simple, easy to apply score that utilizes variables that are readily available at the time of admission to hospital. It can be used to stratify patients into different risk group based on the total score. Apart from a mortality predicting tool, DECAF score can also be used to triage the patients to decide the place of care and level of intervention required during exacerbation of COPD.

Conclusion

DECAF score can be used to predict the outcome of COPD exacerbation. It can help the clinicians to anticipate the morbidity and mortality associated with exacerbation like the length of hospital stay, need for intensive respiratory care and assisted ventilation and the possible final outcome.

DECAF score can also be used in the initial triage of patients and categorize them into different risk groups so that the level of care and management could be individualized in each group. This risk categorization allows early escalation of management strategy in moderate and high risk groups of patients.

Limitations

1) Sample size is only 118. A more extensive study including follow up of patients after discharge could lead to a more accurate result.

2) Lack of comparison with other tools in similar clinical scenario, like APACHE and CURB-65.

3) Study did not address the association of eosinopenia with infective exacerbation and sepsis.

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Case Report

A Neglected case of Chronic Cough

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Introduction

Cough, a distressing symptom both for the patient and the treating physician should be evaluated when conventional treatment fails to produce improvement. Here we describe the report of a young male with cough ignored for almost a year, and diagnosed to have adenoid cystic carcinoma which had local extensive local spread. Adenoid cystic carcinoma, though popularly described as a salivary gland tumor, is the most common primary malignant tracheal tumour. It is notorious for its perineural spread and high rates of recurrence after resection.

Case report

A 25 year old male who had been working as a boat driver presented with progressive dry cough for a duration of 1 year, insidious onset dyspnoea on exertion for 2 months with grade 2 MMRC at presentation, fever and right sided pleuritic chest pain for 10 days, 3-4 bouts of minimal to moderate haemoptysis and noisy breathing. He was not bothered about these persisting symptoms until the development of haemoptysis and noisy breathing. There was no history of associated wheeze, nocturnal exacerbation of symptoms or exacerbation with exposure to dust or cold. No history of loss of appetite or loss of weight. Difficulty in swallowing, change in voice, post nasal drip sensation or acid reflux symptoms were absent. He was a smoker with 5 pack years and an alcoholic with episodic binge drinking.

On examination he was well built and nourished with no pallor, icterus, cyanosis, lymphadenopathy or edema. Bilateral clubbing was present. Stridor was audible on lying supine and to the left. Vitals were stable and he was afebrile. There was tracheomediastinal shift with features of volume loss on the right side. Vocal fremitus, vocal resonance and breath sounds were decreased in the right hemithorax. There were crackles in the right infraxillary and infrascapular areas. Rest of the systems examination were within normal limits. Clinically we made a diagnosis of right lung collapse secondary to intraluminal obstruction. Considering the age of the patient and clinical findings, the differential diagnosis considered were foreign body aspiration (past history of binge alcohol drinking), hypoplastic right lung, slow growing intraluminal tumour and endobronchial tuberculosis. Routine blood investigations were within normal limits. Sputum AFB was negative. Chest X ray PA (Figure 1) showed collapsed right middle and lower lobe with ipsilateral shift of trachea and mediastinum, hyperinflation of left lung and displacement of the anterior junctional line to the right side. There was cut off in the right main bronchus. The flow volume loop on spirometry showed significant flattening of the expiratory portion of curve with preserved peak. (Figure 2) Corresponding CECT Thorax mediastinal window showed same findings with a lobulated growth in the lower end of trachea extending into the right main bronchus and extra tracheal spread (Figure 3 and 4) Fibreoptic bronchoscopy (Figure 5) showed a large lobulated glistening mass lesion occupying the lower part of trachea occluding the right main bronchus. The

scope was passed distal to the lesion on deep inspiration. The carina was also infiltrated with tumour and there was almost near total occlusion of the right main bronchus. Biopsy from the mass revealed adenoid cystic carcinoma.

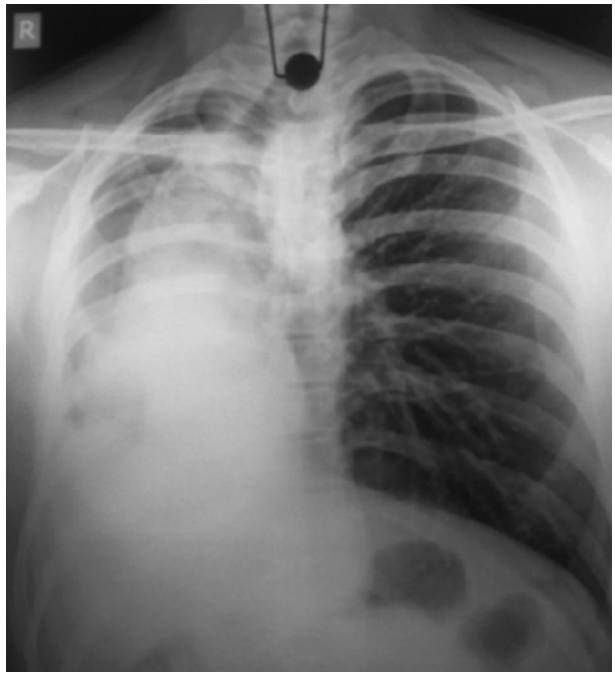

Figure1 – Chest Xray showing collapsed right middle and lower lobes with cutoff in right main bronchus and compensatory hyperinflation of the left lung.

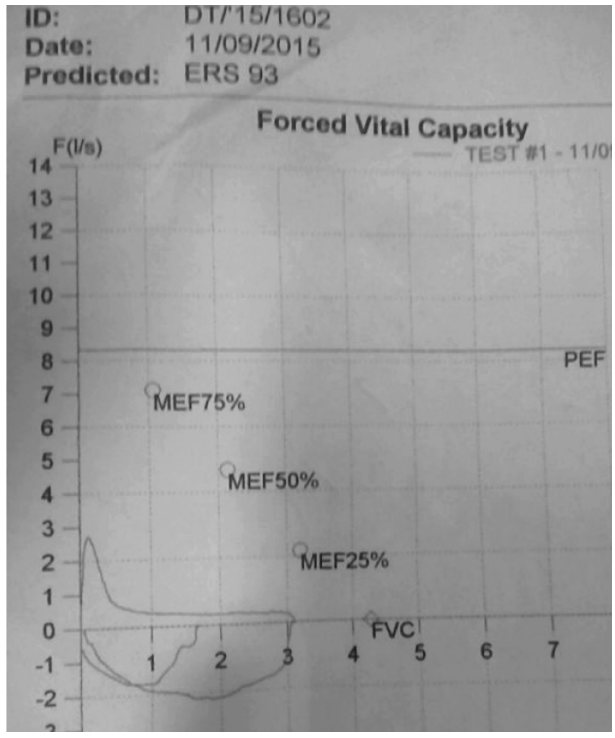

Figure 2 – Flow volume loop showing flattening of the expiratory portion.

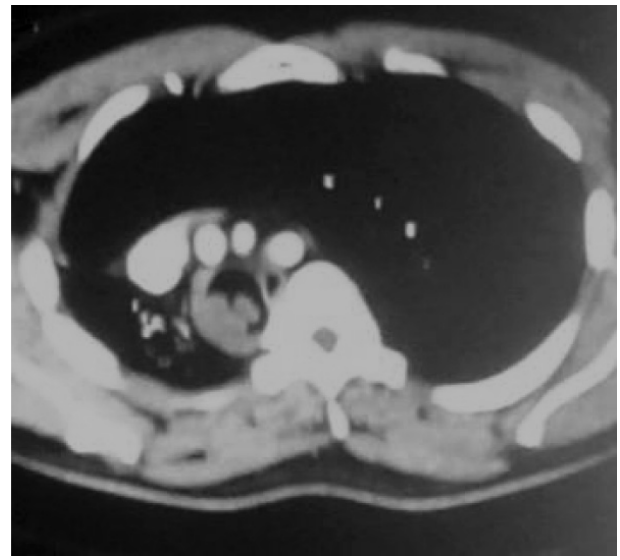

Figure 3–CT Thorax axial cut with intra tracheal polypoidal mass

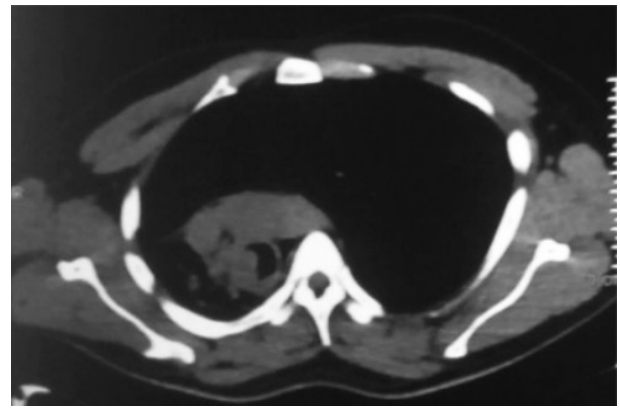

Figure 4 – showing mediastinal extension of the intratracheal mass

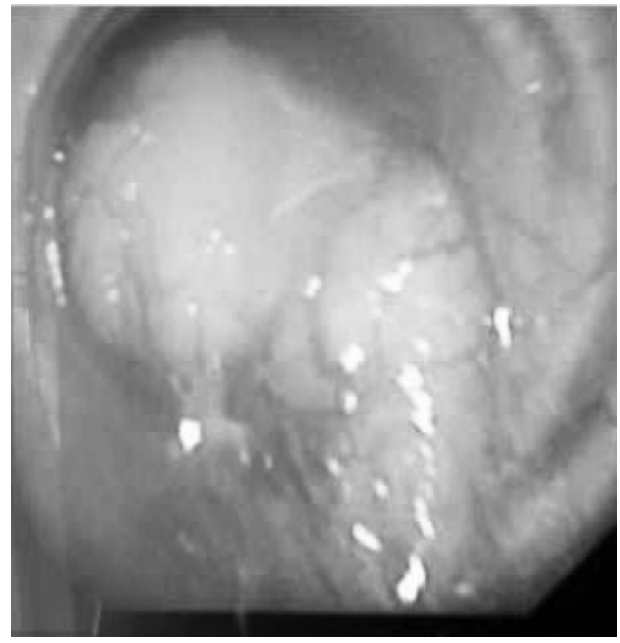

Figure 5 – Intra tracheal mass found at Fibreoptic bronchoscopy.

Since there was no evidence of lung involvement, extrapulmonary metastasis or mediastinal involvement, and surgery being the treatment of choice, he was referred to the department of cardiovascular and thoracic surgery. As expected, perioperatively it was found that there was extensive lesion in right main bronchus and lower part of the trachea with infiltration of tracheal wall. Right pneumonectomy with resection of 4 cm of trachea was done in order to get an adequate margin clearance. Thereafter a tracheal reconstruction was attempted, but under tension due to inadequate length of the stump. The immediate post op period was uneventful. But the histopathological examination of the resected specimen showed inadequate margin clearance of the tumour. While being planned for post operative radiotherapy, he had an episode of massive hemoptysis and he succumbed to death . An irritant cough which had been neglected for a year had a tragic end.

Discussion

Adenoid cystic carcinoma (ACC) is the most common tumor of minor salivary glands in head and neck region. In the lungs it is thought to arise from ductal/ myoepithelial cells of bronchial submucosal glands.[1] Centrally located ACC arises in the trachea or mainstem bronchi as in our case and presents as an exophytic endobronchial mass causing obstructive symptoms. Overlying mucosa is often grossly normal. Peripheral ACC is uncommon. Males and females are equally affected. Cases have been reported worldwide from 20 years to 82 years. Perineural invasion is common. Cough, dyspnoea, haemoptysis, wheeze, and stridor are frequent presenting symptoms.

Morphologically it can be Nodular, Flat or Mixed (nodular and flat). Being flat in growth indicates a more infiltrative growth into the adjacent structure. Histopathologically there are 3 types-Tubular, cribriform and a solid patern. Characteristic feature is "Mucinous cysts" which are present within the tubular and cribriform patterns. The Adenoid cystic variety with tubular pattern has the best prognosis, cribriform pattern has intermediate and solid pattern has the worst prognosis. The most common site of disseminated disease is the lung parenchyma, but extra thoracic metastases are also reported.

Surgery remains the most effective management. Long-term survival can be achieved with adequate resection. But adenoid cystic carcinoma with its early perineural spread, causes difficulty to the surgeon in attaining a complete and adequate resection. Local recurrence may occur even as late as 10 yrs following resection.[2] These tumors are extremely radiosensitive. Hence postoperative radiotherapy to enhance local control is recommended for T3&4 tumors, incomplete resection, bone involvement, perineural invasion, high-grade tumour, and recurrence. Palliative radiation is used for local control of the disease when surgery is contraindicated as in distant metastasis. To date, adjuvant chemotherapy has not been considered efficacious for either cure or prolonged survival. The 5-year survival for adenoid cystic carcinoma is reported as 91% after early detection and treatment.[3] In adenoid cystic carcinoma, retreatment of locally recurrent disease yields prolonged survival. Aggressive local therapy for recurrent disease is indicated if the probability of long-term survival is high.

Mucoepidermoid carcinomas are another similar tumor arising in mainstem bronchi or the proximal lobar bronchi. Common age of presentation is in the 3rd to 4th decade. The right bronchial tree is more commonly affected in children. Patients present with symptoms of obstruction like wheeze, cough, dyspnoea or stridor due to a polypoid endobronchial mass. Bronchoscopic biopsy is diagnostic. Histopathological examination should demonstrate a combination of mucin secreting goblet cells, squamous cells and intermediate cells. Three histologic grades have been defined based upon mitotic index, cell atypia and necrosis. Low-grade mucoepidermoid tumors mostly present in children and young adults, usually without hilar involvement, and have a 5-year survival ranging from 70% to 80%. High-grade tumors have a substantially worse prognosis, with a 5-year survival of only 30% to 45%. High-grade tumors are more common in adults and may invade adjacent structures, lymph nodes, vascular, and perineural spaces. Complete resection with mediastinal lymph node dissection is the treatment of choice. Some may respond to EGFR tyrosine kinase inhibitors.[5] Prognosis is correlated with the grade of the lesion, the existence of nodal involvement, and the success of the initial surgical resection. Incomplete resection is more likely in high-grade tumors and postoperative chemoradiation may be necessary for such cases. High-grade tumors are uniformly fatal in 11 to 28 months.

Acinic cell tumors (Fechner tumors) are usually found in the salivary glands, and hence a diligent

search for an extrathoracic primary is essential when they are diagnosed from thoracic tissues. They can be central, endobronchial,or even peripheral unlike the former ones. Symptoms vary according to the location of the lesion. Microscopically a pattern resembling a neuroendocrine tumor is seen and may need to be differentiated from the more common carcinoid tumor. Acinic cell tumors are slow-growing and recurrence or metastases after complete excision has not been reported.

Slow growing tumours of the trachea can present as chronic cough or wheeze and mimic the common diagnosis of Asthma. So if a patient complains of persisting cough or wheeze in spite of adequate therapy, an intraluminal lesion should always be kept in mind as a differential diagnosis even if the chest radiograph appears normal.

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Case Report

A case of Hyperhomocysteinemia due to Vitamin B12 Deficiency Presenting as Massive Pulmonary Embolism

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Abstract : 25 years old female presented with dyspnoea, syncope, tachycardia and tachypnoea. On examination she had elevated JVP with clear lung fields and loud P2. Chest Xray showed bilateral prominant hilum, ECG - S1Q3T3 with right ventricular strain pattern. Echocardiography showed right atrium, right ventricle dilatation with positive McConnell's sign and mild PAH.A Doppler study showed partialy thrombosed right proximal popliteal vein and CTPA- Right and Left main pulmonary artery massive thrombosis. All investigations to detect etiology for thromboembolism showed negative except markedly raised serum homocysteine level, which prompted us to search for the cause and was found to have vitamin B12 defficiency. Such an association of deficiency of vitamin B12 leading to hyperhomocysteinemia and subsequent thrombosis of popliteal veins and presenting as massive pulmonary embolism though rare, is documented in literature.

A 25 years old lady was admitted to our ward with complaints of dyspnoea and syncope. She had complaints of exertional dyspnoea for the past 2 months, she is an Ayurvedic Doctor and was taking some medicine for the same. She is a strict vegetarian and was known to be anaemic since her adolescence and was on dietary correction. Her last Hb was checked one month prior to admission and it was 11.9g/dL. She gives no history of any prolonged or severe respiratory symptoms in the past. No history suggestive of any bleeding/clotting disorders, malignancy or oral contraceptive pill intake. No history of any surgery, prolonged immobilization or any recent prolonged travel. There was no history of any bleeding disorders, congenital abnormalities or sudden death in the family. She is a strict vegetarian, avoids milk and milk products.

On admission she was drowsy, pale looking, afebrile, in respiratory distress, heart rate-125/mt, blood pressure- 80 systolic, respiratory rate- 40/mt and saturation by pulse oxymetry was 70% in room air.JVP was 8 cm above the sternal angle. On auscultation- Air entry was bilaterally equal over all areas, no added sounds. S1 S2 heard, loud P2 over the pulmonary area, no murmurs. She was admitted to Critical Care Unit and started on Non invasive ventilation, IV fluids and Noradrenaline infusion.

Chest X ray was showing bilateral hilar prominance (Figure 1), ECG - S1Q3T3 (Figure - 2) with right ventricular strain-pattern.

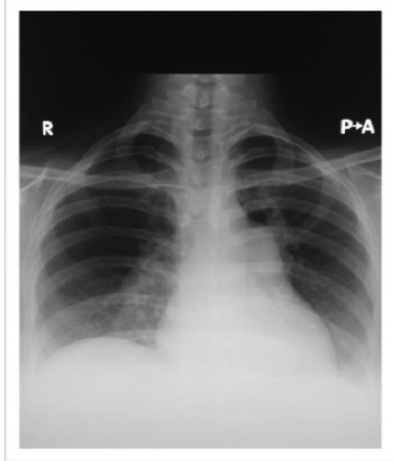

Figure 1 : Chest X ray showing bilateral hilar prominance

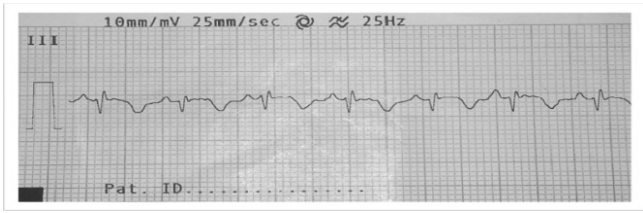

Figure 2 : ECG showing Q3T3 in lead III

Echocardiography showed dilated right atrium and right ventricle with positive McConnell's sign and mild Pulmonary artery hypertension (Figure - 3)

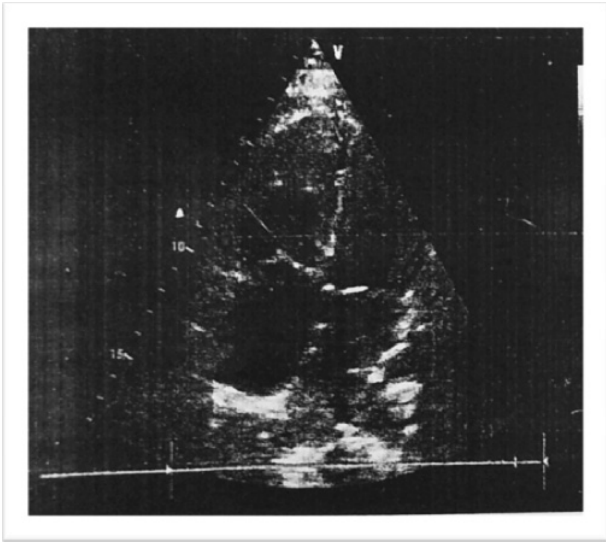

Figure 3 : Echocardiography showing dilated right atrium and right ventricle.

CT Pulmonary Angiography showed Right and left main pulmonary artery massive thrombosis. (Right> left) and extending to right lower and left upper branches (Figure - 4)

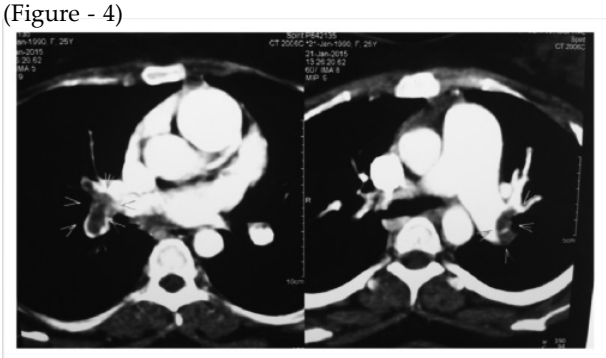

Figure 4 : CT Pulmonary Angiography showing massive thrombus in right and left main pulmonary artery

Doppler leg veins was showing partially thrombosed Right proximal popliteal vein. Her D-dimer was more than 10000ng/ml [normal<500ng/ml] and NT-proBNP- 4093pg/ml [normal<125]. She was immediately thrombolysed with Tenecteplase40 mg intravenous bolus. Her other blood investigations were Hb-11.8 g/dL, TLC-10500 cells/mcL, platelet count- 2,50000/cumm, ESR-45mm/hr, Peripheral smear- Dimorphic anaemia, TFT-WNL, Blood urea-21mg/dL, S.Cr- 0.8mg/dL, S.Na-135mEq/L, S.K-3.76mEq/L, SGOT/PT- 27/105 U/L, S.Ferritin- 23 [13-150], TIBC- 359 [250-400], S.Fe- 39 [37-145]. Case was worked up for the causes of thrombosis,causes of hypercoagulable states were checked. ANA- negative, AntidsDNA- negative, Factor 5 Leiden mutation– not detected, APLA- negative, Protein C - 83 [55-140], Protein S- 113 [55-123], Antithrombin III– 99 [70-122], Serum Homocysteine-65mmol/l (3.7 – 13.9), Urine homocystiene-negative. Factors which leads to homocysteinemia were checked, Vitamin B6- 74 [30 - 144 nmol/L] , Vitamin B9 [Folate] -20ng/ml [3-20], Vitamin B12- 80 pg/ml (211- 911). Patient was anticoagulated with Inj low molecular weight heparin, NIV and oxygen. Patient was shifted to ward,put on methyl cobalamine, folic acid and iron supplementation. She was discharged on oral anticoagulation, 2 weeks after discharge she is symptomatically better on T. warfarin with an INR - 2.56 and venous doppler of lower limb showed no evidence of thrombosis.

Discussion

Homocysteine was discovered in 1932 by Butz and du Vigneaud.Over the last 30 years, a growing body of evidence has documented the role of hyperhomocysteinemia as an independent vascular risk factor. However, the mechanisms through which elevated circulating levels of homocysteinecause vascular injury and promote thrombosis remain elusive. Most findings have been achieved in vitro of studies employing exceedingly high concentrations of Homocysteine, whereas only a few studies have been carried out in vivo in humans. In homocystinuric patients, homozygotes for mutations of the gene coding for the cystathionine beta-synthase enzyme, abnormalities of coagulation variables reflecting a hypercoagulable state, have been reported[8]. In 1964 Mudd and collegues reported the absence of cystathionine beta-synthase activity in the liver of a subject with homocystinuria. In 1994 Falcon et al reported hyper homocyteinemia as a risk factor for thrombosis occurring before the age of forty[10]. A meta-analysis of ten case-control studies was done by Martin et al in 1998 and found hyperhomocysteinemia as a risk factor for vascular thrombosis[11].

Apart from hereditary factors, there are certain acquired factors[7] which can influence and can associate with raised levels of plasma homocysteine like:-

1. Vitamin B12 and folic acid deficiency.
2. Drugs: Metformin, methotrexate, phenytoin, carbamazepine, niacin, theophylline.
3. Diabetes mellitus, renal failure, hypo thyroidism.
4. Rheumatoid arthritis, SLE, psoriasis etc.

Kang and co-workers[4] have classified hyperhomocysteinemia as follows[5,6]:

1. Moderate Risk: 15-30 μmol/L
2. Intermediate Risk: 30-100μmol/L
3. Severe Risk:> 100 μmol/L

Here in this case report, the patient falls into Intermediate riskgroup (Kang classification). Our patient presented with a constellation of symptoms and signs, which were highly suggestive of pulmonary embolism due to deep veinthrombosis that had developed because ofhyperhomocysteinemia secondary to vitamin B12 deficiency. However, with treatment in the form of vitamin B12, folic acid supplementation and anticoagulation, patient recovered completely. Early recognition and prompt search for the etiology can not only save the patients, but also can prevent further recurrence of thromboembolic phenomenon.

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Case Report

Rare Presentation of Renal Cell Cancer

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Introduction

Lytic lesions in the chest wall bony cage mandates a search for a malignancy that could be either within the lung or hidden elsewhere. Here we report a case where the chest wall involvement in the form of rib erosion turned out to be metastatic spread from renal cell cancer (RCC).

Case Report

A 66-year-old male farmer presented with unsteadiness in walking for one month. He had a fall followed by a few episodes of non-projectile vomiting four days back. There was no history of seizures or loss of consciousness. He also gave a history of dull aching pain in the right lower lateral aspect of chest and cough with scanty mucoid expectoration for three weeks. He did not have symptoms suggestive of chronic respiratory illness or any other co morbidities. He was a smoker with a smoking score of 800.

Physical examination revealed clubbing. His chest movements were reduced in lower part of right hemithorax. Percussion revealed dull note on right infra axillary area with local tenderness. Breath sounds were reduced in the same area on auscultation. Abdomen was soft with no organomegaly. Higher mental functions and cranial nerves were within normal limits. Lower limbs had a power of grade 4 and sluggish deep tendon reflexes. Cerebellar signs were negative.

He was initially evaluated from Neurology department with CT head (fig1) and MRI (fig 2) and referred to the Pulmonology Department for further work up after chest radiography (fig 3).

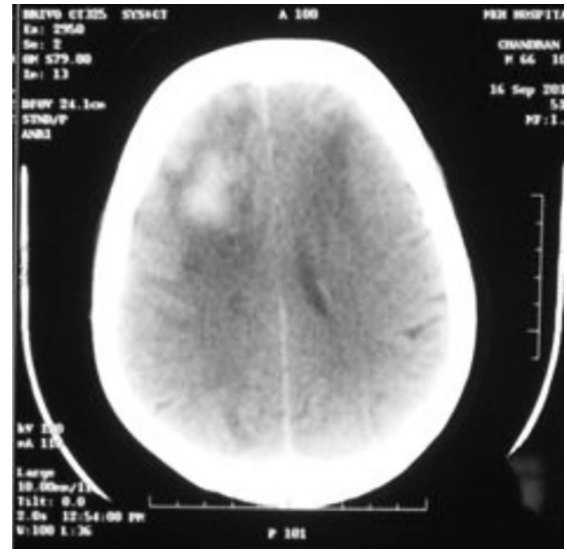

Figure 1 : CT head showing a well defined hyperdense lesion with irregular margins in right frontal lobe with surrounding edema and mass effect

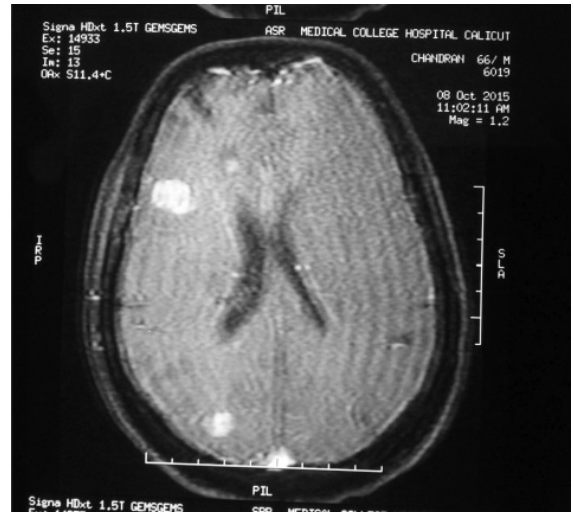

Figure 2 : MRI showing multiple enhancing hemorrhagic lesions involving bilateral cerebral hemispheres with perilesional edema suggestive of metastasis.

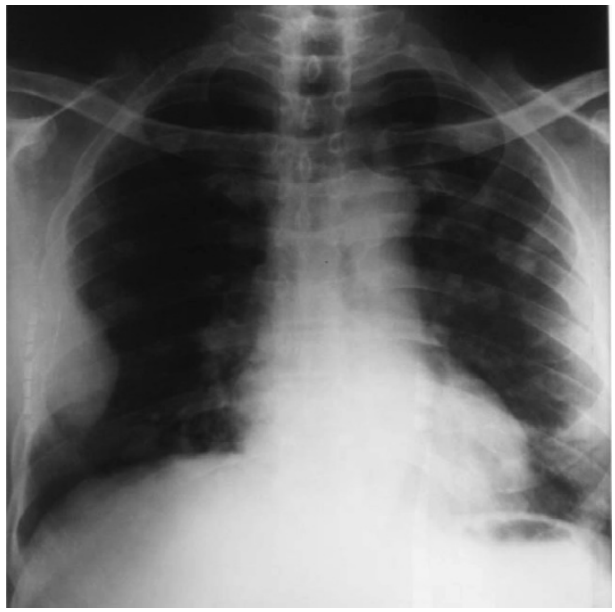

Figure 3 : Chest Xray showing pleural based opacity in the right mid and lower zones with multiple rib erosions. Multiple nodular lesions present bilaterally.

Fibre-optic bronchoscopy was within normal limits. Contrast enhanced CT of the thorax showed a heterogeneously enhancing soft tissue density lesion (10.8 X 5.7cm) in right lateral chest wall with intrathoracic extrapleural extension and destruction of seven, eight and nine ribs on the right side at the junction of anterior and middle thirds. There were bilateral multiple soft tissue density lesions of varying sizes in lung parenchyma.Enlarged prevascular (9.8 mm), right upper paratracheal (7.5 mm) and lateral aortic lymph nodes (10 mm) were also present. A right-sided pleural effusion was present.Available abdominal slices revealed a heterogenously enhancing soft tissue density lesion involving mid and lower pole of right kidney of size 7.4 X 6.4 cm.

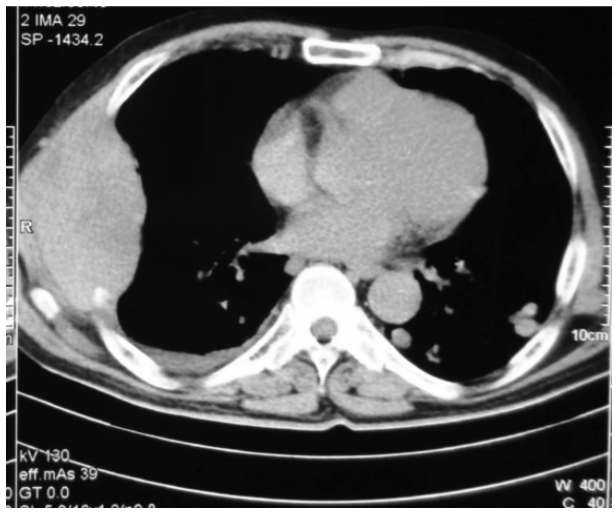

Figure 4 : CT Thorax showing soft tissue density lesion in right lateral chest wall with rib destruction and minimal effusion

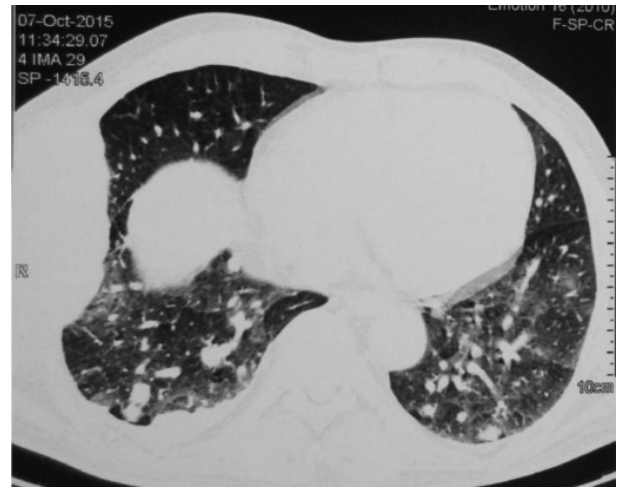

Figure 5 : CT lung parenchymal window showing bilateral multiple soft tissue density lesions of varying sizes with patchy ground glassing

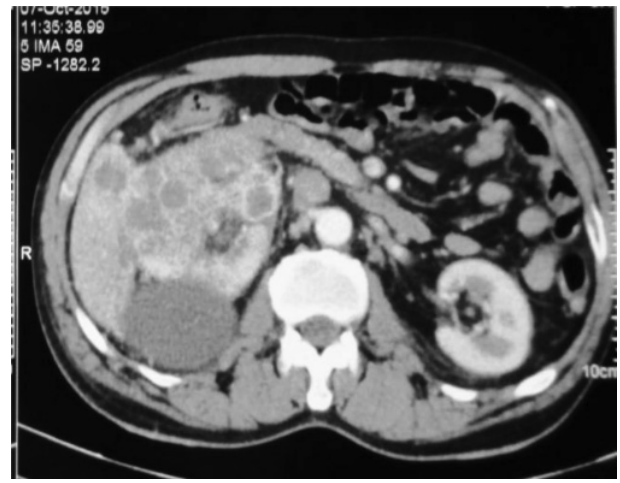

Figure 6 : CT abdominal cut showing a heterogenously enhancing soft tissue density lesion in right kidney

Thoracic ultrasonography showed an area of mixed echogenicity in the right infra axillary region with absence of lung sliding. 20 ml of hemorrhagic pleural fluid was aspirated from the anechoic area. Analysis showed it to be an exudate with Total Count of 2600 cells/mm^3 and lymphocyte predominance (76%). Adenosine deaminase level was 126 IU/L and cytology was negative for malignant cells.

Trucut biopsy was done under ultrasonographic guidance from the chest wall lesion and histology turned out to be metastasis from renal cell carcinoma. The patient was sent for further oncologic and palliative care.

Discussion

Renal cell carcinoma originates within the renal cortex and approximately 30% of patients present with metastasis[1]. 50%–60% of patients with metastases

have lung involvement.Metastasis usually occurs by hematogenous spread to the parenchyma of other organs. For patients with lung metastases, hilar or mediastinal lymph node involvement occurs in 22%–30% of cases and is associated with worse outcomes[2,3].

In advanced RCC with bone metastases,the most common sites of bone involvement are pelvis and ribs (48%), followed by the spine (37%)[4]. Contrary to the pattern in some other tumour types such as prostate cancer, bone metastases from RCC are predominantly osteolytic and associated with bone destruction.

Nowadays more than 70% of all renal cancer cases are detected as incidental findings on imaging studies obtained for unrelated reasons[5]. The classical teaching that renal cancer presents with signs and symptoms such as hematuria, flank pain and palpable mass is more an exception rather than the rule. Extensive thoracic metastasis from renal carcinoma with parenchymal, chest wall, mediastinal node involvement and pleural effusion is less common. Apart from the rarity of this case, the clinical point that we would like to highlight is that the patient may not always have noticeable respiratory symptoms in spite of widespread metastasis to the lungs.

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Case Report

Pulmonary Cryptococcosis: A Rare Presentation

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Abstract : Cryptococcal infection is a systemic fungal infection which is usually described in literature in association with immunosuppression. We describe a case report in which Cryptococcal infection was identified in an immunocompetant individual with pulmonary as well as rib involvement. Vertebral involvement has been described for Cryptococcal infection but rib involvement is a rare entity.

KEYWORDS : Cryptococcal infection, retronegative, rib involvement.

52 year old female presented to our out patient department with cough and fever of 10 days duration. Cough was productive with purulent expectoration, and fever was intermittent in nature. She was treated in a peripheral hospital following which fever subsided. She gave history of right sided pleuritic chest pain as well. There was loss of appetite and loss of weight.

On examination, her vitals were stable. She had a diffuse thyroid swelling and a 3x2 cm nontender swelling in the lower and posterior aspect of the right side of chest. There was no lymphadenopathy and lower respiratory system examination was within normal limits.

Hematological examination revealed an ESR value of 30mm in the first hour. Mantoux was 15mm. Sputum AFB was negative and viral markers (HIV, HBsAG, anti-HCV) were negative. Thyroid function tests were within normal limits. Chest X ray (fig 1) showed a homogenous nodular opacity involving the right lower zone corresponding to the right 8th rib posteriorly with widened carina and minimal blunting of right costophrenic angle.

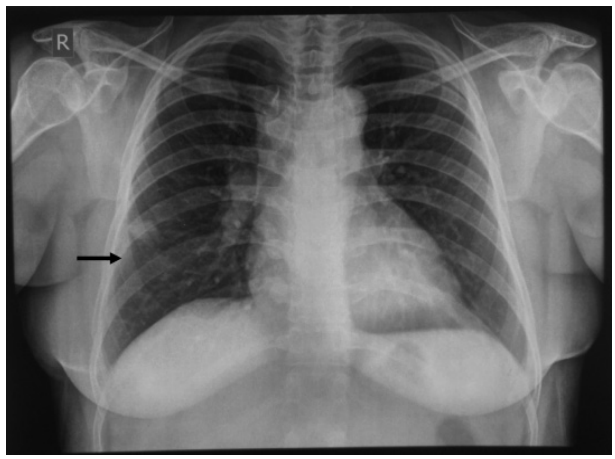

Figure 1 : Chest X ray at the time of presentation- showing Right lower zone nodular opacity, mediastinal widening, minimal blunting of right costophrenic angle

CECT Thorax (fig 2) showed a round to oval enhancing lesion involving right lower lobe with no cavitation or calcification.

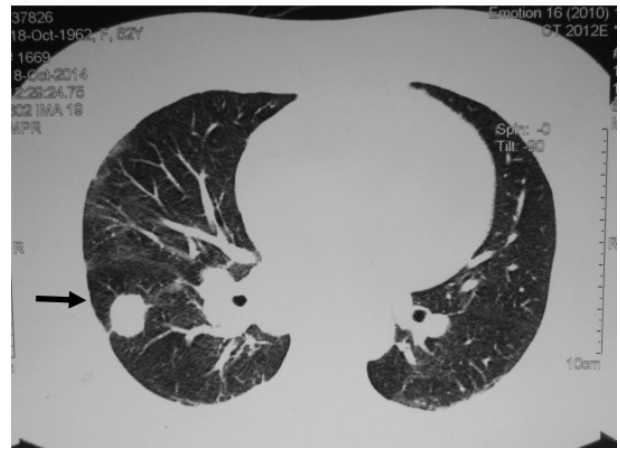

Figure 2

Multiple enlarged mediastinal lymph nodes including paratracheal, hilar and prevascular nodes were noted. Bilateral pleural thickening was present with a lytic lesion in the posterior part of 8th rib on right side (fig3).

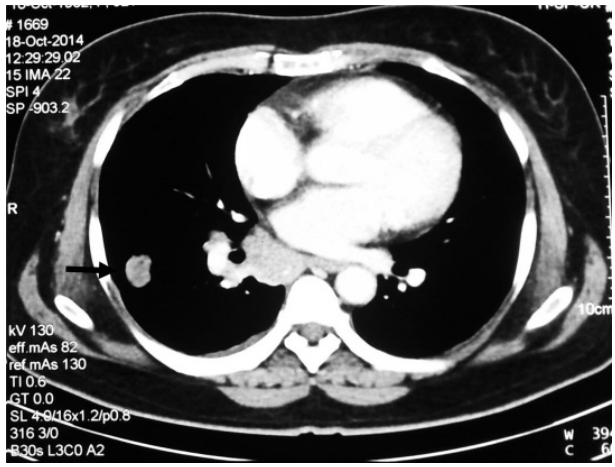

Figure 3

We proceeded with a fine needle aspiration from the chest wall swelling. AFB smear done from the aspirate was negative and cytological studies did not reveal atypical cells, granulomas or malignant cells. Thyroid swelling FNA was reported as lymphocytic thyroiditis. Flexible bronchoscopy was within normal limits. Due to the presence of lytic lesion, Serum electrophoresis was done which was within normal limits. Bone scan (fig 4) showed lytic lesion on the right posterior 8th rib with a suspicion of malignancy.

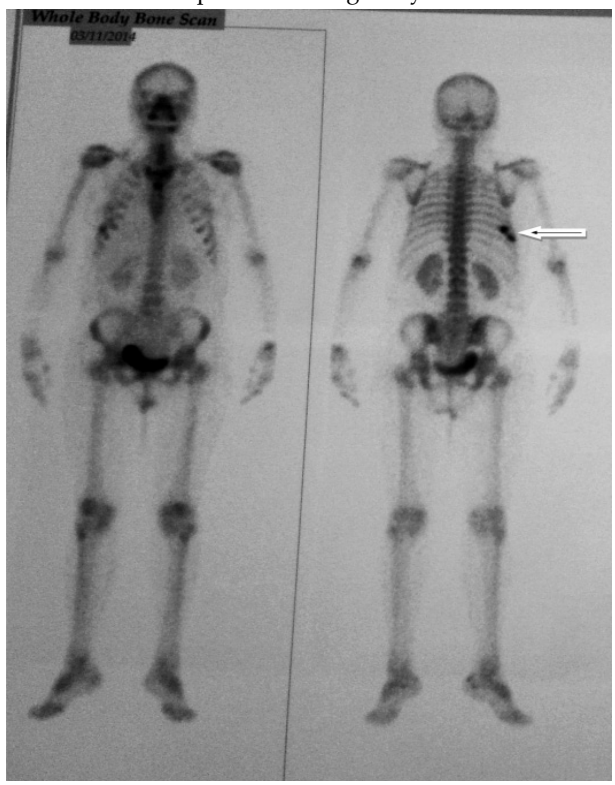

Fig 4-Bone Scan showing lytic lesion right 8th rib posteriorly

In order to obtain a histopathological diagnosis, rib (rightposterior 8th rib) excision biopsy was done. Pathological report came as Cryptococcal infection rib, positive for hematoxylin and eosin stain (fig 5).

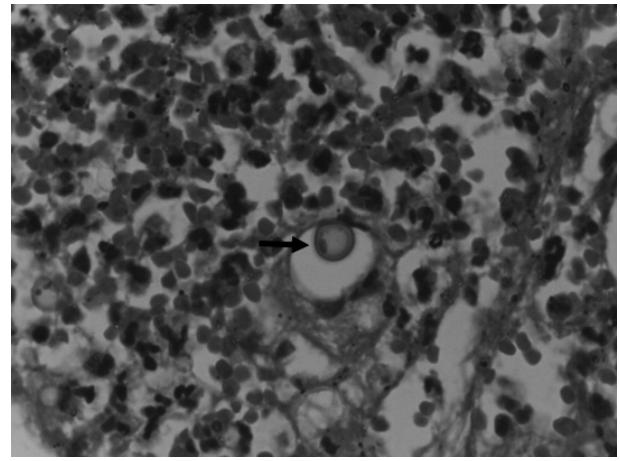

Fig 5-Cryptococcal Capsule – Hematoxylin and eosin stained rib excision biopsy slide with features of infection- presence of neutrophils, hemorrhages seen

Periodic acid Schiff stain (fig 6)

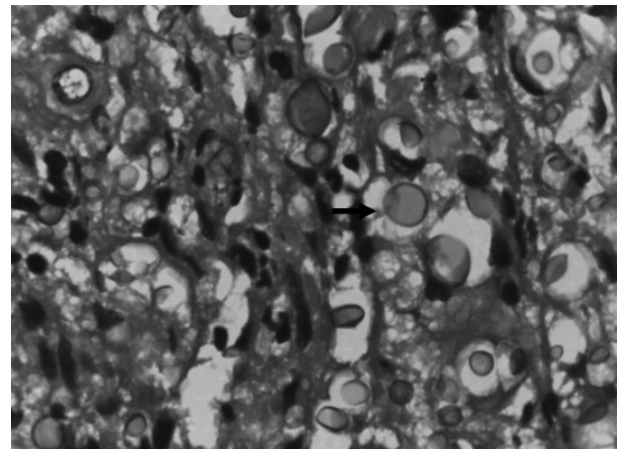

Fig 6 - Cryptococcal capsule in Periodic acid Schiff reagent stained rib excision biopsy slide

Gomorri methanamine silver stained slide (fig 7)

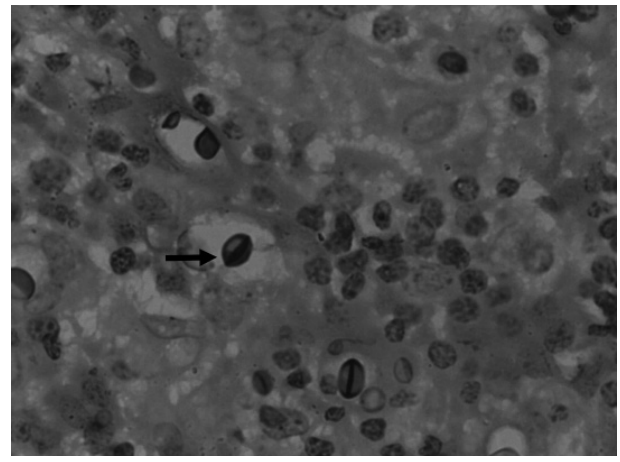

Fig 7-Cryptococcosis- Gomorrimethanamine silver stained rib excision biopsy slide

Mucicarmine stained slide (fig 8)

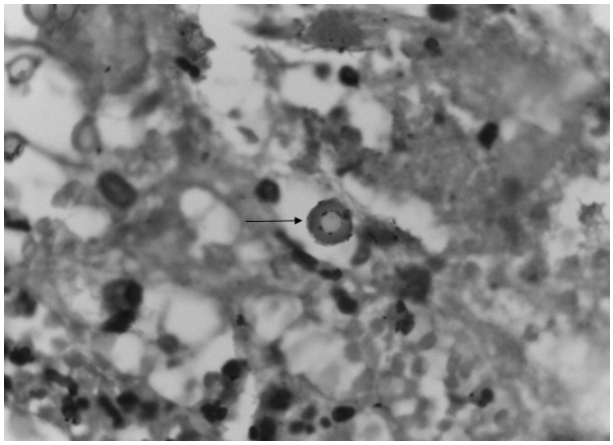

Fig 8 - Cryptococcal infection- rib excision biopsy slide stained with mucicarmine

Neuro medicine consultation was done. Cerebrospinal fluid study was within normal limits. CT Brain did not yield any positive findings. On our further probing in order to identify the cause of this infection, we came to know that the high altitude area, where our patient was residing had thick growth of eucalyptus trees. She is staying in that area for the past 30 years. Serotypes B and C classified as Cryptococcus neoformans var. gatti are more common in tropical and subtropical regions in association with eucalyptus trees rather than avian droppings.

With suspected lung involvement and biopsy evidence of rib involvement and with no features to suggest brain involvement, patient was started on fluconazole(as per IDSA Guidelines) and kept under follow up. 2 months later on her review she was symptomatically much better and there was clearance of the non homogenous opacity in repeat Chest X Ray (fig 9).

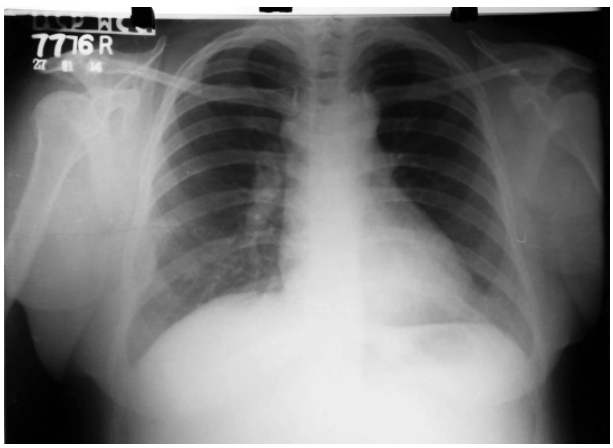

Fig 9-Chest X ray after 2 months on follow up. Clearance of right lower zone opacity seen with a soft tissue swelling (post rib excision biopsy)

Patient is kept under close follow up.

Discussion

Cryptococcosis is caused by infection with the encapsulated fungus *Cryptococcus neoformans*, an organism with a worldwide distribution. Inhalation of *C. neoformans* initiates the infection in the lungs with hematogenous dissemination most often involving the meninges. The spectrum of disease ranges from asymptomatic pulmonary infection in the immunocompetent host to diffuse pulmonary disease associated with respiratory failure and widespread disseminated disease in the immunocompromised host. Four serotypes of C. *neoformanshave* been described-A, B ,C ,D.Serotypes A and D predominate in North America and Europe and grow best in composted bird droppings or rotten vegetation. Serotypes B and C are classified as C. *neoformans* var. *gatti* and are more common in tropical and subtropical regions in association with eucalyptus trees rather than avian droppings. The disease also appears to be more frequent in diabetics. C.neoformans var. gatti infection occurs mostly in immunocompetent hosts[1].Concerning the clinical features of pulmonary cryptococcosis in immunocompetent hosts, there is awide variety of presentations. In a review by Campbell, symptoms may be absent, or may present as cough (54%), chest pain (46%), increased sputum production (32%), fever (26%), weight loss (26%) and haemoptysis (18%)[2].

In immunocompetent patients, chest imaging of cryptococcosis can reveal the presence of pulmonary abnormalities, but the findings are non-specific and inconclusive. Chest radiographic features range from single pulmonary nodule to widespread nodular and airspace consolidation[3]. Rarely, they can also present as cavitation, pulmonary infiltrate, hilar and mediastinal adenopathy, and pleural effusion. Under computed tomography, the most common finding was pulmonary nodules, whereas lymphadenopathy, consolidation, pleural effusion and cavitation are uncommon[4]. There are many overlapping radiological features among pulmonary cryptococcosis, lung cancer, pulmonary tuberculosis, bacterial pneumonia and other mycosis.The diagnosis of pulmonary cryptococcosis is based upon histopathologic detection of the organism at lung biopsy or a positive culture from the respiratory specimen. Serum cryptococcal antigen plays an adjunctive role, it is usually positive in cases of disseminated disease or in case of immunosuppression[5].

The treatment of cryptococcosis depends on the immune status of the host and the clinical manifestations. Mild to moderate disease limited to the respiratory system is usually treated with fluconazole in both immunocompetent and immunosuppressed individuals. Well selected immunocompetent patients may be observed for evidence of spontaneous remission, although recent guidelines suggest treating even those who are asymptomatic. Patients with severe disease, central-nervous-system involvement, or any other evidence of dissemination are treated with an "induction" regimen of amphotericin B and flucytosine followed by "consolidation" fluconazole6. According to IDSA guidelines, if CNS disease is ruled out infection occurs in single site, fungemia not present, and with no other immunosuppressive risk factors, consider fluconazole (6 mg/kg) orally for 6-12 months7. Our patient was started on fluconazole and kept under close follow up as majority of relapses occur within the first one year of disease. In the repeat Chest X ray which was taken after 2 months of follow up there was clearance of the nodular opacity.

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Acknowledgement

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Case Report

Pulmonary Multi Nodular Disease of Rare Etiology

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Abstract : A case of Alveolar soft part sarcoma (ASPS) in a 30 yr old female who presented as multi nodular disease is reported due to its rarity.

Key words : Alveolar soft part sarcoma, multi nodular disease, metastasis lung

Introduction

Alveolar soft part sarcoma is a rare soft tissue sarcoma that usually occurs in young females. It account for 0.5- 1% of all sarcomas. Typically ASPS arises in muscles and deep soft tissue of the thigh or the leg, but can also appear in the hands, neck, and head. The prognosis is poor and is often characterised by late metastases to lung and brain. The case of a 30 yrs old female presented with multi nodular metastasis in the lung from a primary ASPS is reported due to its rarity.

Case report

30 year old female referred to our department from district TB centre for evaluation of chest x-ray abnormality. She presented with cough with mucoid expectoration and breathlessness of six months duration. She had no haemoptysis or chest pain but had significant weight loss and loss of appetite. She gave history of excision of a swelling in the right lower neck one month ago. No other significant past history. Clinical examination showed grade1 clubbing and anemia. Respiratory system examination was normal except few scattered crepitations. Her routine blood examination showed Hb 10g%, normal blood counts, RBS 116mg%. Chest X-ray (fig.1) showed multiple large nodular opacities more confluent in the mid and lower zones. A CT Chest taken demonstrated multiple nodules of varying sizes (fig.2) .Few lesions had the size of a mass. Nodules were of random distribution with feeding vessel sign in some of the nodules. There was no significant enlarged lymph node or pleural effusion. A CT guided lung biopsy was done and it showed lung with a circumscribed neoplasm composed of cells arranged in an alveolar and organoid pattern. Nests of cells were having abundant eosinophilic cytoplasm and large vesicular nuclei with fibrovasculaar septae in between. Scattered mitotic figures and areas of haemorrhage were noted. Immunohistochemistry was done and neoplastic cells were negative for cytokeratin, CD10, thyroglobulin and synaptophysin. PAS positive material was noted in the cytoplasm of the neoplastic cells (fig.4). The morphological features and immunofindings confirmed the diagnosis of alveolar soft-part sarcoma. The slides and blocks of the previous neck swelling were reviewed and it showed the same neoplasm. Considering the two, the

final diagnosis of Alveolar soft part sarcoma with lung metastasis was made. The patient was referred to oncologist for further management.

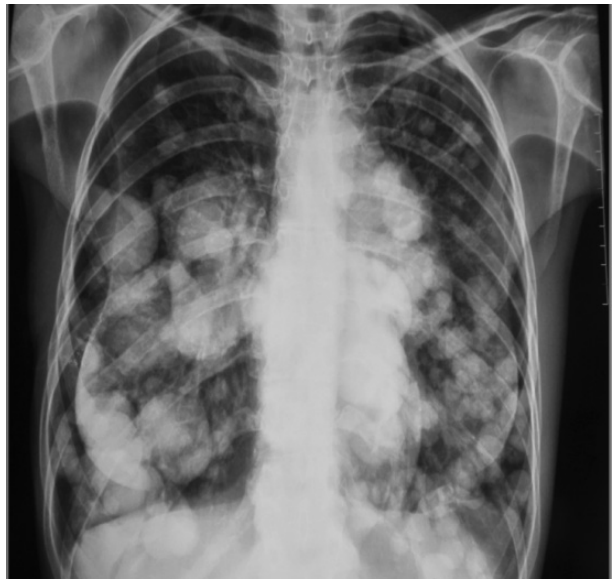


Fig.1 : Chest x-ray showing multiple varying size nodules and masses

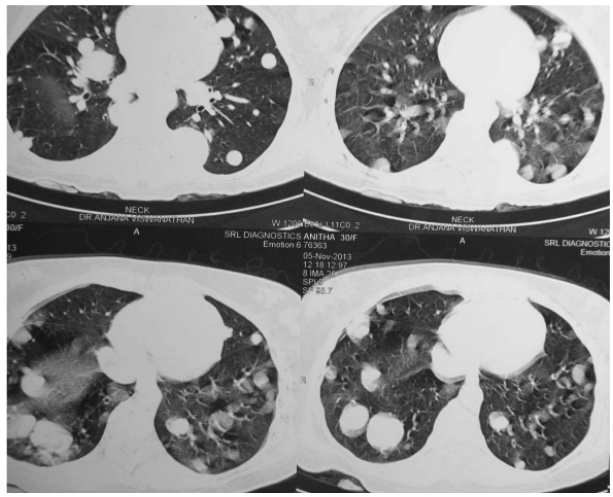


Fig.2 : CT Chest showing multiple nodules in random distribution and masses

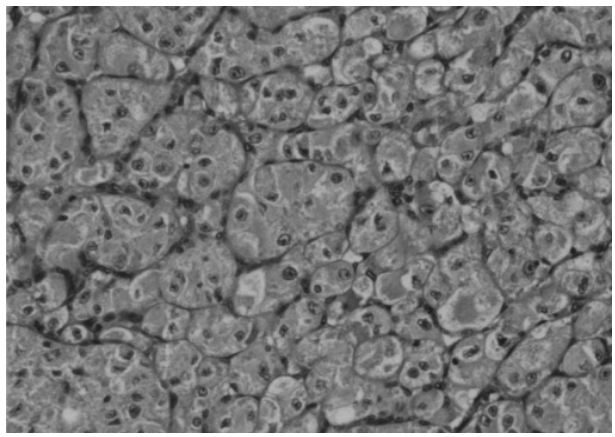
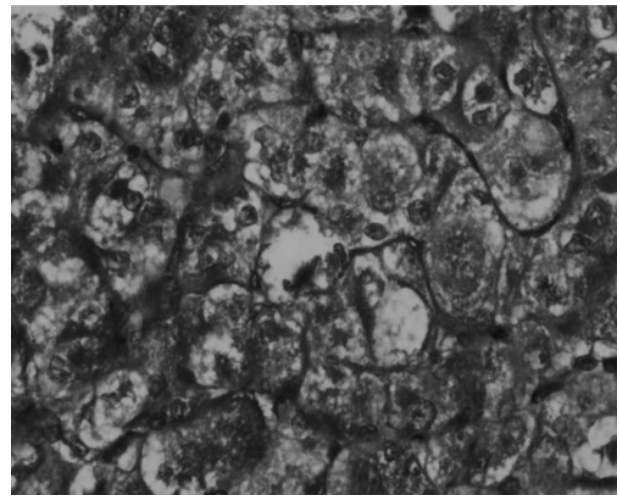


Fig.3 : H&E section 200X showing cells with abundant eosinophilic granular cytoplasm in alveolar pattern



F ig. 4 : PAS staining 400X showing PAS positive cytoplasmic crystals

Discussion

Alveolar soft part sarcoma (ASPS) is a rare, poor prognostic neoplasm of unknown histogenesis with a distinctive histology, specific molecular characteristics and unique clinical behaviour. ASPS is an un common tumour typically occurs in adolescent and young adult patients[1]. As many as 60% of ASPS cases are seen in females and this female predilection is not found in children[2]. ASPS usually presents as a soft, painless, slowly growing mass that rarely causes functional impairment. In adults the lower extremities are the most common location. But it has been reported in other sites like female genital tract, mediastinum, breast, urinary bladder, GI tract and bone[3]. In children ASPS most commonly occur in the head and neck region. These tumours are extremely vascular, and occasionally present as a pulsatile mass with an associated bruit. Despite a relatively indolent tumour growth pattern, up to 79% of the patients develop metastasis. The most common metastatic sites are lung ,bone, central nervous system, and liver[4]. Because of the relative lack of symptoms, in many patients the tumour is easily overlooked and metastasis to the lung or other sites may be the first disease manifestation[5]. Accurate diagnosis and treatment of this unusual tumour requires a high index of clinical suspicion coupled with clinicopathological correlation via appropriate radiographic studies. If the clinical or radiographic interpretation is equivocal, early biopsy is essential to differentiate alveolar soft part sarcoma from arteriovenous malformation. Radical resection is the therapy of choice for localized disease[6]. Despite the occurrence of metastases in up to 79% of patients, 5-year overall survival rates range from 45 to 88% [7]. No

significant survival advantage have been achieved by utilizing conventional chemotherapy, radiation or excision for patients who have evidence of metastasis at the time of original diagnosis as compared to patients who are not treated[4]. Our patient had a swelling in the neck which was excised one month prior to her visit and that could be the primary. The lung nodules which showed the same histopathological features are metastasis from the primary ASPS lesion. In view of the refractory nature of ASPS to conventional chemotherapy she was kept under strict follow up.

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